

Adolph Weil (1848–1916)

Kindly supplied by Dr E. Ashworth Underwood, The Wellcome Historical Medical Museum, London

Frontispiece

LEPTOSPIROSIS
IN MAN AND ANIMALS

BY

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FOREWORD BY

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F. & S. LIVINGSTONE LTD.
EDINBURGH AND LONDON
1958



E & S LIVINGSTONE LTD, *March* 1958

PRINTED IN GREAT BRITAIN

FOREWORD

THE leptospiral aetiology of Weil's disease was established forty one years ago The laboratory proof of clinical belief had been long awaited as it was away back in 1886 that Weil had given his reasons for separating this condition from the ruck of bilious fevers, and Landouzy apparently, had to some extent anticipated this by some three years When proof came it was not the work of a single individual or group of investigators Credit for it is usually given to the team of Japanese investigators led by Inada, but two groups of German workers studying the outbreaks of jaundice which were occurring in their troops on the Western front in the 1914-18 war, had independently concluded that Weil's disease was caused by a spirochaete It is true that different names were given to the spirochaetes incriminated, but it was clear that they were dealing with the same micro-organism, the one now known by the name of *Lep*

of

of

the leptospires and the diseases they engender has not waned Not that this has led to my working afresh on any of these problems, but I have attempted to keep myself informed as to what was transpiring in the leptospiral world And had I not seen the monograph to which I am writing this foreword I might have retained the comfortable belief that I had succeeded reasonably well However let me confess that I had no idea how rapidly and how far knowledge of the leptospires and the leptospiroses had advanced I was aware, of course, that in the past forty years a number of new leptospiral diseases of man had been described—mud fever, seven-day fever, autumn fever and canicola fever to mention some of them—and that their causal leptospires had been shown to be distinct from *L icterohaemorrhagiae* But I had no idea that over 50 different pathogenic leptospires had been described, 37 of which had definitely been associated with disease in man The great majority of these leptospires have already been given specific

names by their discoverers, but Dr Alston and Dr Broom consider that this is prejudging the issue and very wisely prefer to refer to them as "serotypes" until a closer study shows whether or not they are entitled to specific status

It would appear also that the various human diseases caused by these numerous serotypes do not differ as much as one might expect from the fact that they bear different labels They have a family likeness due to a basically similar disease pattern Where they do differ is in severity, and this is quite definitely a matter of serotypes since some, *L canicola* for example, consistently produce a mild type of disease in man, whereas others, like *L icterohaemorrhagiae*, give rise to a more serious condition with a not inconsiderable mortality Incidentally, man is no more than an accidental host of these leptospires—he is of no oecological significance for them And with the possible exception of the dog the same probably applies to the other large animals in which infection has been found, the true hosts are provided by various species of Muridae

Many other aspects of leptospirosis might be mentioned, but sufficient has probably been said to show how much would be gained by bringing all this information together into one comprehensive story And who could be better equipped to do this than the two authors of this monograph who, by their personal researches, have added so materially to our knowledge of leptospirosis

S P BEDSON

PREFACE

Our aim in this book has been to tell what is known about leptospires and the diseases which they cause in man and animals, and to show where new knowledge is at present being gained and may be hoped for in the future Recently, in the U S A , in Malaya, in Israel and in parts of Europe, the disease has been found with increasing frequency, and often caused by serotypes which had not previously been known to be present in those regions

It is a matter for concern that, though in human beings the majority of cases are of the milder forms, the disease may cause an illness which affects people singly or in groups, and may require several weeks of convalescence Also it has been recognised—only during recent years—that endemic leptospirosis among farm animals may cause serious financial losses in some countries

We have written this book in historical sequence and have given full references for the statements made We have included epidemiology, clinical and pathological descriptions of the disease, therapeutics and prevention of infection Since we wish to consider the subject as comprehensively as possible, we are glad that Mr Charles Doughty, Q C , has written a Chapter on the legal aspects of leptospirosis in England and Wales He gives details of the legal responsibility of employers towards any of their employees who contract the disease by reason of the nature of their work, and also gives examples of other sets of circumstances in which persons might be held legally responsible for infections contracted on their premises

Acknowledgments

We acknowledge with pleasure our gratitude to friends and colleagues for advice, information and criticism—especially Miss M F Crowley, B Sc , Mr I L Martin, M R C V S , Dr M D Milne, Dr T St M Norris and Dr Charles Wilcocks, Editor of the Bulletin of Hygiene For portraits of the pioneers we are indebted to Dr E Ashworth Underwood (Weil), Professor M Kitaoka (Inada), Sir James Kilpatrick (Noguchi) and Professor A Charlotte Ruys (Schuffner) Dr J D Fulton

and Dr D F Spooner generously provided the hitherto unpublished electron micrographs for Figs 4, 5, 6 and 7 We have acknowledged the sources of the other Figures in the captions, but take this opportunity to thank the Authors, and the Editors of the Journals and Publishers who gave us permission for reproduction

We are grateful to Dr L J M Laurent and the Editor for allowing us to make use of articles published in *The Lancet* for some of our descriptions of illustrative clinical cases The names of the Authors who kindly allowed us to reproduce Tables from their published works are shown in the captions, but we wish also to record our thanks to the Editors of the Journals or the Authorities responsible for the publications in which they appeared, namely —*Rendiconti dell'Istituto Superiore di Sanita* (Table I), *Documenta de Medicina Geographica et Tropica* (Table II), *Proceedings of the Royal Society of Medicine* (Table VI), Walter Reed Army Institute of Research (Table XIII), *Journal of the American Medical Association* (Table XVIII) *Schweizerische Medizinische Wochenschrift* (Table XXII), *Journal of Clinical Pathology* (Table XXIII), *British Medical Journal* (Tables XXIV and XXV), *The Lancet* (Tables XXVI and XXVII), University of Illinois (Tables XXVIII and XXIX), *The Veterinary Record* (Table XXXI), *Medical Journal of Australia* (Tables XXXII and XXXIII)

We owe many thanks to Miss Patricia Wright for preparing the typescript and for suggesting a suitable subject for the book jacket, and also to Mr W J Bishop for preparing the Index Finally we must express to our Publishers our appreciation of all the help they have given us in preparing this book

J M ALSTON
1958 J C BROOM

CONTENTS

SECTION I

MORPHOLOGY, CLASSIFICATION, PHYSIOLOGY AND DISTRIBUTION OF LEPTOSPIRES

SECTION II

LEPTOSPIROSIS IN MAN

GENERAL CHARACTERS OF LEPTOSPIRES

MORPHOLOGY, CLASSIFICATION PHYSIOLOGY AND DISTRIBUTION

CHAPTER I

HISTORY AND GENERAL SURVEY

Clinical Observations Discovery of the Infective Agent Creation of Genus *Leptospira*, Taxonomy of *Leptospira icterohaemorrhagiae* Discovery of Further Serotypes Distribution and Spread

HISTORY

Clinical Observations

The separation of leptospiral jaundice from other infectious diseases of the liver occurred in two stages—the first was clinical, and the second, nearly thirty years later, bacteriological The term 'Weil s disease' was first used by Goldschmidt (1887) to designate the form of infectious jaundice which Adolf Weil (1886), Professor of Medicine at Heidelberg, had established as a separate entity from a study of four cases Two of the cases had occurred in 1870 and the other two in 1882, but they all presented such similar features that Weil considered them to be the same disease All the patients were men, and in each case there was a febrile illness with *severe nervous symptoms*, enlargement of the liver and spleen, jaundice and signs of renal involvement After a severe but relatively short course recovery was rapid Recurrence of fever for five or six days, following an afebrile period of one to seven days, occurred in three patients

In differential diagnosis, Weil carefully considered the recognised diseases of (1) catarrhal jaundice, (2) primary nephritis, (3) a combination of catarrhal jaundice and primary nephritis, (4) yellow atrophy of the liver, (5) pyaemic or septicaemic fever, (6) recurrent fever, (7) bilious typhoid fever He suggested that the cases represented a new entity, although he could not demonstrate either its anatomical basis or the infective agent The four patients were employed respectively as a chemist, soldier, merchant and waiter—occupations which gave no clue to the aetiology of the condition However, Landouzy

(1883 a & b), who had in fact described the disease three years before Weil, associated it with work in sewers He noted that men were most likely to be affected when they had been employed in sewer work for a comparatively short time, but he attributed the disease to emanations from sewage, and thought that in time the men developed an immunity He described the illness in one patient as a general disease affecting the lungs, spleen and liver, with albuminuria, and in a second as a disease chiefly of the lungs, liver and kidneys

There seems little reason to doubt, as stated for example by Dupre (1891) and Austoni (1953), that cases of Weil's disease had been recorded by still earlier observers Some of the claims however are almost certainly incorrect Thus there is no evidence that the epidemic of jaundice in Minorca mentioned by Cleghorn (1751) was Weil's disease, and the very fatal malady which attacked soldiers wounded at the siege of Cairo in 1802 (Larrey, 1812) was most probably gas gangrene

During the thirty years following the publication of Weil's article, the term 'Weil's disease' was used in all parts of the world, but particularly in Germany, to describe a febrile illness with jaundice in epidemic and endemic form, but there was doubt as to its applicability to individual cases and even to its justification in general

As early as 1889, Young reported as an example of Weil's disease an illness which began two days after the patient had returned from Yeomanry training in South-West England Young gave an excellent day-to-day description of the disease, noting suppression of urine, tarry stools, jaundice, injection of the eyes, and recovery after five days of acute illness In 1892, Jaeger reported nine cases among soldiers in the garrison at Ulm, Germany, and attributed their illness to bathing in a river near the town

In 1903, Chowdry gave a good account of what he considered to be Weil's disease in endemic form at Port Blair in the Andaman Islands He had records of 588 cases which occurred between 1892 and 1903, and he associated the illness with sudden or prolonged exposure to rain while cutting wood, or working on embankments, in brick fields or rice fields, 13 per cent of the patients died, but relapses were uncommon in those who survived

Hunter (1908) stated that most French and English writers had declined to recognize Weil's disease as separable from *icterus gravis* or infectious jaundice, and he agreed with that opinion However, he gave a detailed account of the characteristics of the disease, derived mainly from German reports This included the clinical features given in Weil's own report, and Hunter laid emphasis on the severity of muscular pains and nephritis enlargement of the spleen was said to be usual Among the aetiological features he noted that the incidence was highest among boys and young men—by some estimates 90 per cent of the patients were male Most were men whose occupations or habits exposed them to insanitary surroundings Hunter quoted nine cases among men working in a slaughterhouse near Dresden, in addition to the nine mentioned above (Jaeger, 1892) due to bathing in a river

Cockayne (1912), in a good review, clearly separated epidemic catarrhal jaundice from infectious jaundice or Weil s disease He believed that the former was much more common and milder, was almost world-wide and was an air-borne infection He considered that Weil's disease was probably due to contaminated water or food, and occurred chiefly around the Mediterranean Sea He based his opinion about the Mediterranean area on reports such as that of Sandwith (1904), who described an endemic and epidemic illness of severe jaundice, often with nephritis, anuria and uraemia, in Smyrna and Alexandria Sandwith however did not consider that Weil's disease was a separate entity

In 1915, Boggs was equally sceptical when he wrote in an article in Osler and McCrae's *System of Medicine*

> Weil s disease is a type of infectious jaundice in which fever, enlargement of the spleen and liver, nephritis, and muscular pain accompany the icterus But there are so many similar sporadic and epidemic cases in which one or more of these features may be wanting that it seems hardly justifiable to separate this group sharply from all the others

This scepticism was shown to be unjustified by the discovery of the causative organism of this form of jaundice in 1915, and the failure in clinical acumen seems to be rather conspicuous when the description of the illness and the aetiological features enumerated by Hunter are considered It would appear that

the success of bacteriology had made European clinicians doubtful of their judgment

DISCOVERY OF THE INFECTIVE AGENT

Japanese clinicians and pathologists had been convinced of the existence of Weil's disease as a separate form of jaundice long before the discovery of the infective organism Inada, Ido, Hoki, Kaneko and Ito (1916) asserted that the illness was well known in both endemic and epidemic form, and that it was characterized by conjunctival congestion, muscular pain, fever, jaundice, haemorrhagic diathesis, albuminuria, and had a fairly high death rate They stated also that splenic enlargement was uncommon and occurred in only 10 per cent of their patients

In November 1914, at the Imperial University in Kyushu, Inada *et al* (1916) first saw spirochaetes in the liver tissue of a guineapig which had been injected with blood from a patient suffering from Weil's disease They obtained similar results with blood from 13 out of 17 other cases of Weil's disease, and failed to find spirochaetes in guineapigs inoculated with blood from patients suffering from other infections, including catarrhal jaundice They concluded therefore that the spirochaete was the casual organism of Weil's disease, and they named it *Spirochaeta icterohaemorrhagiae*

The results of other experiments with the spirochaete were included in the same communication They found that cultures in artificial medium grew better at 22°–25°C than at 15° or 37°C They showed that the organisms could gain entry into guineapigs through the abraded or even the apparently intact skin They noted that living spirochaetes could be seen in patients' urine for as long as twenty-five days after the onset of illness By means of Pfeiffer's test they showed the presence of antibodies in the serum of patients, and found that the immune bodies persisted in the blood for a number of years

Ido Hoki Ito and Wani (1916) reported finding virulent *S icterohaemorrhagiae* in the kidneys of 40 per cent of 86 house and ditch rats By disinfecting ground and removing stagnant water from certain places in coal mines, they considered that they had twice prevented epidemics of the disease They experimented with active and passive immunization in man and

animals, but did not achieve convincing success by preventive inoculation

The causative organism was discovered independently at a later date by German workers and was named by Hubener and Reiter (1915, 1916) *Spirochaeta nodosa*, and by Uhlenhuth and Fromme (1915, 1916 a & b) *Spirochaeta icterogenes* These names are not used currently in English, but are regarded as synonymous with *Leptospira icterohaemorrhagiae*

Hübener and Reiter reported that they had infected guineapigs with the peripheral blood of at least seven men suffering from Weil's disease and noted that they were most successful when blood was taken in the first three to six days of illness The disease produced in guineapigs showed the signs of Weil's disease seen in human beings, and caused death on the fifth to twelfth day, with the morbid anatomy and histology of the human disease They made twelve serial passages in guineapigs, infecting the animals *per os* and *per anum* They also successfully infected monkeys and rabbits From the urine of a patient on the fifteenth day of illness they produced the disease in guineapigs Later, by dark-ground illumination, they saw small, elongated structures showing lashing motility, and believed that these were the cause of the disease

Uhlenhuth and Fromme also transmitted the infection from man to guineapig and were first in Europe to demonstrate active spirochaetes in dark-ground preparations from guineapig livers The same organisms were seen in fixed films stained by Giemsa's or Levaditi's methods

The Japanese findings were confirmed by British workers in soldiers infected in France Stokes and Ryle (1916 a & b) diagnosed spirochaetal jaundice on a clinical basis in ten soldiers, and they demonstrated spirochaetes in guineapigs successfully inoculated from 2 out of 10 Stokes, Ryle and Tytler (1917) reported about a hundred cases, and they infected guineapigs with blood taken from the second to the seventh day of illness They showed the presence of protective antibodies in the serum of convalescents, but they failed to culture the organism They also demonstrated spirochaetes in guineapigs inoculated with material from wild rats

Dawson and Hume (1916) gave an account of 178 soldiers suffering from infectious diseases with jaundice They

separated them into 76 with spirochaetal jaundice, and 102 diagnosed as enteric jaundice or catarrhal jaundice Of the spirochaetal type (mainly diagnosed clinically) 18 were severe and 58 mild; splenic enlargement was uncommon. They passed the infection to a guineapig from at least one patient, and saw what they took to be spirochaetes in the blood and urine of others Dawson, Hume and Bedson (1917) in a summary of their work in Belgium on 76 cases gave a low mortality of 4 to 5 per cent Costa and Troisier (1916 a) reported the infection in French troops, and Sisto (1917) and other workers found it on the Italian front

CREATION OF GENUS LEPTOSPIRA

Noguchi (1917, 1918 a & b) carefully studied the *Spirochaeta icterohaemorrhagiae* of Inada, strains from British cases of Weil's disease in Flanders and from wild rats in the U S A He found that these organisms were the same in form and in immunological tests, and that they resembled no other organism except *Spirochaeta biflexa* which Wolbach and Binger (1914) had isolated from a fresh-water pond in Massachusetts, U S A Noguchi considered the morphology to be sufficiently characteristic to justify the creation of a new genus which he named '*Leptospira*', to be included in the Order Spirochaetales along with *Spirochaeta*, *Saprospira*, *Cristispira*, *Spironema* and *Treponema*

Babudieri (1954 b) suggested dividing the Order Spirochaetales into two families and eight genera on the basis of optical and electron microscopical studies The division was based on the presence or absence of septa axostyles, undulating membranes, flagella, cristae, and granules of volutin This classification is given in Table I

The generic name *Leptospira* has become more and more firmly established since 1917 The original strain of *L biflexa* found by Wolbach and Binger did not survive its first subculture and, although the name is used for nonpathogenic saprophytic leptospires, *Leptospira icterohaemorrhagiae* is the type 'species' or 'serotype' of the genus It has been shown that saprophytic leptospires which are readily obtained from tap water and water in natural surroundings differ culturally (Hindle, 1925) and serologically from pathogenic serotypes



FIG. 1

Ryokichi Inada (1874-1950)

Kindly supplied by Prof. M. Kitaoka, National Institute of Health, Tokyo, Japan



Hideyo Noguchi
June 11, 1923

FIG 2

Hideyo Noguchi (1876 1928)

Kindly supplied by The Dean The London School of Hygiene and Tropical Medicine London

TABLE I

SUBDIVISION OF THE ORDER SPIROCHAETALES

(after Babudieri 1954 b)

Septa	Family	Axostyle			No.		Genus
Septa *present*	Family Saprospiraceae		Undulating membrane *present*		1	Genus	*Cristispira*
			Undulating membrane *absent*		2		*Saprospira*
Septa *absent*	Family Spirochaetaceae	Axostyle *present*	Flagella *present*		3	Genus	*Spirella*
			Flagella *absent*	Volutin *present*	4		*Spirochaeta*
				Volutin *absent*	5		*Leptospira*
		Axostyle *absent*	Crista *present*	Flagella *present*	6	Genus	*Treponema*
				Flagella *absent*	7		*Cristispirella*
			Crista *absent*		8		*Trichospira*

TABLE I

SUBDIVISION OF THE ORDER SPIROCHAETALES

(after Babudieri 1934 b)

Septa *present* Family Saprospiraceae		Undulating membrane *present*		1 Genus	*Cristispira*
		Undulating membrane *absent*		2	*Saprospira*
Septa *absent* Family Spirochaetaceae	Axostyle *present*	Flagella *present*		3 Genus	*Spirella*
		Flagella *absent*	Volutin *present*	4	*Spirochaeta*
			Volutin *absent*	5	*Leptospira*
	Axostyle *absent*	Crista *present*	Flagella *present*	6 Genus	*Treponema*
			Flagella *absent*	7	*Cristispirella*
		Crista *absent*		8	*Trichospira*

From Indonesia, *L pyrogenes* was first reported in 1923, and *L bataviae* in 1926 Both of these have been found to cause disease in Eastern Asia and elsewhere

In 1928, the isolation of *L grippotyphosa* was made in the U S S R This serotype is carried by field rodents, and is a very frequent cause of leptospirosis in human beings and animals in many countries

In 1931, *L andaman A* was identified in human beings in the Andaman Islands and has only rarely been found elsewhere

The isolation of *L canicola* in the Netherlands was reported in 1933, and this serotype which is pathogenic to dogs, cattle and human beings has been found in most parts of the world

In 1937, the identification of *L australis A* and *L australis B* was reported from Australia These serotypes are the cause of leptospirosis in sugar-cane workers in Australia and in some persons in other countries Also in 1937, *L pomona* was reported from Australia, and this serotype infects human beings and animals, especially cattle, in many parts of the world

L hyos (syn *mitis* Johnson) was first reported in 1942 in Australia and has been found, like *L pomona*, to infect pigs and human beings in several countries

Three new serotypes were first reported from Denmark and have later been found in other countries, chiefly European, they infect field rodents and human beings They are *L sejroe* (1939) *L saxkoebing* (1944) and *L ballum* (1944)

The discovery of new serotypes still continues In 1954 and 1955 several have been found in Australia, Malaya and Borneo In U S S R several serotypes have been isolated, but have not yet been compared fully with serotypes known elsewhere More than 50 serotypes have been named although not all have yet been compared fully with the 'recognized' standard serotypes The serological comparison of 39 is given in Table II

Distribution and Spread

Leptospirosis is transmitted to man, generally indirectly, from small rodents and certain other animals which harbour leptospires in their kidneys and excrete the organisms in the urine For most serotypes rats and field mice are the reservoir

TABLE II

LYSIS TITRES OF

oom 1954 with add tions)

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DISTRIBUTION AND SPREAD

Leptospirosis is transmitted t from small rodents and certain leptospires in their kidneys and urine For most serotypes, rats a hosts of prime importance, and r

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CHAPTER II

MORPHOLOGY AND CLASSIFICATION OF LEPTOSPIRES

Dark field Microscopy Electron Microscopy Stability of Antigenic Constitution Antigenic Subtypes

MORPHOLOGY

Dark field Microscopy

All serotypes of leptospires show the same morphology For a description as seen microscopically using light of the visible part of the spectrum it would be difficult to improve on the account given by Wolbach and Binger (1914) of *L biflexa* These workers studied the organism alive and dead by dark ground illumination and also in films fixed by osmic acid and stained by Giemsa s method For the exact observation of morphology in as unimpaired a state of the organism as possible and for photographic studies they exposed drops of fluid containing leptospires to the fumes of osmic acid and then rapidly mixed them with 3 per cent melted agar in water on a warm microscope slide Their description of appearances by dark ground illumination is as follows

This spirochete is characterized by the extreme closeness of the turns its small size and curved or flexed extremities The average length is from five to seven microns The amplitude or width of the spirals measured from crest to crest is from [illegible] to 2[illegible] micron

The two ends of the spirochetes are thinner than the body and taper to points They are more or less sharply curved and sometimes have the form of a crook When alive the spirochete spins with extreme rapidity on its long axis in such a manner that the curved ends give the appearance of solid bulbs or flask like bodies In addition to this rapid rotation about the long axis there is fairly rapid translatory motion in either direction of the long axis The living spirochete is straight and presents the appearance of having a rigid long axis Occasional slightly flexed forms which are motionless have been seen to straighten out and to begin to spin rapidly In stained

The possibility of the existence of viruses (similar to bacteriophage viruses) which could lyse leptospires must also be investigated Much less is known of the details of morphology and of the metabolic processes of leptospires and other spirochaetes than of other bacterial organisms



FIG 4

L icterohaemorrhagiae (× 4 000 approx) Photomicrograph by dark-ground illumination Kindly supplied by Dr J D Fulton and Dr D F Spooner National Institute for Medical Research London

preparations the characteristics of the living organisms are lost The bodies become bent or flexed and the spirals less regular and less closely wound

The curved ends, giving unique appearances to the spirochete in motion, are peculiar to this organism, and for this reason have been utilized in the construction of a name The name *Spirocheta biflexa* is proposed

All serotypes of leptospires show the morphology described by Wolbach and Binger, including the curved end or ends of most individuals in a preparation (Fig 4) There are however some strains which are consistently straight-ended, as is a strain of *L benjamin* which we possess, and as the Jackson strain of *L icterohaemorrhagiae* has become in our hands

Electron Microscopy

During recent years, leptospires have been examined by electron microscopy Morton and Anderson (1943) gained a resolving power of 3 mμ and described the appearance of *L icterohaemorrhagiae* and *L canicola* Their measurements agreed reasonably with earlier observations by the visual microscope, but their most interesting findings were the negative ones that structures resembling flagella, such as were seen on some of the treponemes, were not seen on the leptospires Also, unlike some treponemes, leptospires did not show any granular internal structure or extruded granules In some circumstances a sheath-like structure appeared round individual micro-organisms, but they thought that this might be either an artifact due to washing with distilled water or be related to the cell wall

Electron microscopy has been used to examine the granules often seen in old cultures of leptospires It has been thought possible that these granules might represent a filterable stage which could explain the filterability of the genus noticed in earlier studies of the organism Bessemans, Wittebolle and Baert (1942) isolated such granules by the micro-manipulator and believed them to be capable of developing into normal leptospires when transferred to fresh culture medium, they called the granules 'leptospirogènes' Jakob (1949) described round bodies, up to 50 mμ in diameter, each with a flagellum in 20-day cultures of eight serotypes of leptospires He agreed



FIG 4

L icterohaemorrhagiae (× 4 500 approx) Photomicrograph by dark-ground illumination Kindly supplied by Dr J D Fulton and Dr D F Spooner National Institute for Medical Research, London

Fig 5

L icterohaemorrhagiae (× 15 000 approx) Electronmicrograph, washed organism fixed with osmic acid and shadowed with gold-manganum Kindly supplied by Dr J D Fulton and Dr D F Spooner National Institute for Medical Research, London



FIG. 6

with Bessemans *et al* that the granules are part of a reproductive system and that they have no higher survival value than the usual forms Babudieri (1949 a) studied the same cultures as Jakob and considered that they were contaminated with a filterable organism *Hyphomicrobium vulgare* This question needs more study

Babudieri demonstrated an axial filament (or axostyle) in leptospires by the electron microscope, and this feature has also been clearly shown by Bradfield and Cater (1952) and by Spooner (1955) who has provided the photographs reproduced in Figs 5, 6 and 7 Fig 5 shows *L icterohaemorrhagiae* and demonstrates the morphology similar to that seen by dark ground illumination In Fig 6 the fine axial filament can be seen coiling spirally round the main body of the leptospire which is itself spirally coiled, but much less closely than in dark ground preparations The reason for this difference lies in the dehydration which is needed to make a preparation for the electron microscope

In Fig 7 two axial filaments are more clearly seen, and a fine regular cross striation of the longer filament can be observed The nature of these cross striations, and the question whether they are related to the power of motility of the organism require further investigation The leptospires in Figs 5, 6 and 7 were fixed with osmic acid and shadowed with gold manganum, and the photographs show that the filament is more resistant to this treatment than the main body of the organism

Bradfield and Cater s electron micrographs show the axostyle as a thin filament wound with open coils round the thicker, more closely coiled, spiral body of the organism They made models of each genus of the spirochaetes and the model of a leptospire was made by endowing a 'fibre bundle' with contractility and a core with some elasticity

In the model (Fig 8A) this is achieved by winding a stout elastic band spirally around a piece of rubber tubing which is at first held rigid by a metal rod inside it The elastic band is wound under moderate tension and securely fastened to the tubing by wire every few inches If the metal rod is now removed (Fig 8B) the tension in the elastic band (representing the bundle of fibres) throws the rubber tubing (representing the protoplasmic core) into the form of a spirochaete The final shape of the helix is that in which the forces

resisting deformation in the rubber tube just balance the tension in the elastic band

Breese, Gochenour and Yager (1952) also examined eight serotypes of leptospires by electron microscopy, and their photographs of each serotype clearly show an axial filament in the form of a very thin filament entwined about the main structure and often extending beyond it at both ends It would appear that this thin thread, surrounding the leptospire with wide coils and extending beyond the ends of the bulkier part of the organism, was seen by Timmerman (1927) in preparations stained by Giemsa's method and partly decolourized

Czekalowski and Eaves (1955) produced a step by step disintegration of leptospires by the action of sodium deoxycholate, and the resulting appearances were examined by the electron microscope Washed leptospires were suspended in aqueous solutions of sodium deoxycholate, ranging in concentration from 0 01 to 1 0 per cent After periods varying from 30 seconds to 30 minutes the organisms were fixed with osmic acid, and drops of the suspensions were allowed to dry on the usual collodion-filmed grids and shadowed with chromium Electron micrographs of normal leptospires showed the cytoplasmic element and the axostyle ' wound round each other like a rope of two unequal strands ' From their photographs the authors could not determine whether the filament was within the cellular membrane or outside it When leptospires were treated for short periods with low concentrations of sodium deoxycholate the two elements separated Grooves on the cytoplasmic cylinder showed where the axostyle formerly lay Apparently the axostyle was outside the cellular membrane and was generally released by simple unwinding although occasionally it seemed to have cut through the underlying protoplasmic coils After the filament had unwound it remained attached to each end of the cytoplasmic cylinder where it appeared to penetrate into the substance of the cytoplasm, and there terminated in a more or less spherical knob The [illegible] be interpreted as [illegible] ament might [illegible]

Swain (1955) also studied *L icterohaemorrhagiae* by electron microscopy Preparations, fixed with osmic acid, were made

of organisms (1) suspended in normal saline, (2) after exposure for 2 hours to saline diluted 1/3 with distilled water, (3) after incubation for various periods from 10 to 30 minutes with pepsin, and (4) after incubation for 10 minutes with trypsin

and did not conform to the waves of the organism (in this way the findings differed from those of Czekalowski and Eaves) Both pepsin and trypsin dissolved the leptospires, but the axostyle was relatively more resistant than the protoplasm

In spite of these recent advances in description of the morphology of leptospires, nothing is yet known of the internal structure of the body in the way that cell wall, nuclear material, volutin granules and other granular constituents have been found *in situ* in electron micrographs of sections of various bacilli and cocci

CLASSIFICATION

Wenyon (1926) included the Order Spirochaetales among the protozoa, but most workers now follow Breed, Murray and Hitchens (1948), who consider them to be bacteria This is an important change because zoological differentiation depends essentially on morphology, and there are no morphological differences which could be used to subdivide the genus *Leptospira* It was soon realized however that practical considerations made it necessary to have some means of discriminating between groups of strains which showed dissimilar properties

achieve that aim In distinguishing the two workers made use of differences in (1) guineapigs, (2) the symptoms of disease (3) epidemiology, as evidenced by carrier distribution of the two diseases, and serological tests

Other observers have tried to

basis for classifying the strains of leptospires which have been isolated in increasing numbers from disease conditions in man and animals It seems advisable therefore to compare the relative values of the various criteria

(1) Tests of pathogenicity and cross-protection depend on the mutual reactions of host and parasite Variations in the resistance of hosts of the same species may occur as the result of differences of age, sex or other factors In the same way strains of the same serotype may differ markedly in virulence even when freshly isolated It is unlikely therefore that these tests will give regular and consistent results

(2) To some extent it is possible to divide the clinical forms of leptospirosis into severe and mild depending on whether jaundice is or is not a usual prominent feature in human infections The differentiation is however not sufficiently constant to be of value for taxonomic purposes

(3) There is a general tendency for the leptospires which produce the different forms of illness to be associated each with its own carrier host of election But this is by no means an invariable rule One animal species may act as host to more than one serological type of leptospires, and conversely one serotype may be carried by different hosts

(4) Serological tests provide an indication of the antigenic composition of micro organisms and have long been used as a method of differentiation of bacteria The serological characteristics of leptospires might therefore be utilized as a basis for classification, provided that the antigens are stable and distinctive characters

STABILITY OF ANTIGENIC CONSTITUTION

Although most observers consider that the agglutinogenic characters remain constant, aberrancies in the serological reactions of certain well-established 'type strains' have been reported on various occasions Wolff and Broom (1954) studied closely the records of these aberrant results, and reached the conclusion that they did not necessarily indicate that spontaneous antigenic variation had taken place As alternative explanations they suggested that in some cases mistakes in labelling might have been made at some point in a series of subcultures In other instances the cultures might have been

accidentally contaminated with water leptospires which overgrew and finally replaced the original strain

These possibilities emphasize the need for exercising strict precautions to ensure that all cultures are correctly designated, and also the value of periodic verification of the serological characteristics of strains

Wolff and Broom concluded that, provided strains of guaranteed lineage are used, the system based on the antigenic constitution of leptospires provides a valid practical method of classification In view however of the present lack of information about the bionomics of leptospires they considered that it is not possible for a decision to be made regarding the criteria which may finally be used in the subdivision of the genus In these circumstances it does not appear justifiable to designate a group of strains of the same antigenic constitution as a 'species' They suggested that the term 'serotype' (serological type) should be adopted instead

The individuality of the serotypes is established on the evidence provided by cross-absorption tests If, in such reactions, each strain removes all the antibodies from the antiserum of the other strain, the two may justly be considered serologically identical If however only part of the antibody content is absorbed in each case, some 'limit of absorption' must be adopted to allow the decision to be made whether or not the two strains shall be regarded as belonging to the same serotype In defining their standard Wolff and Broom stated that 'two strains are considered to belong to different sero types if, after cross-absorption with adequate amounts of heterologous antigen, 10 per cent or more of the homologous titre regularly remains in each of the two antisera'

The decision to establish the 10 per cent limit was made for reasons of practical expediency, and no theoretical basis is claimed for it Some observers (World Health Organisation, 1956) considered it too high and advocated a lower limit, but at present it is the criterion generally adopted because it appears to work satisfactorily in practice

Even if the 10 per cent limit is retained for establishing separate serotypes, a study of minor antigenic differences *among strains of the 'same' serotype might provide information* of practical as well as theoretical value For example the

TABLE III

LEPTOSPIRES NAMED BUT NOT FULLY INDENTIFIED

Leptospires Never Cultivated for Identification

Name	*Reference*	*Origin*
bilio hemoglobinuriae	Blanchard & Lefrou (1922)	From case of blackwater fever in Belgian Congo Isolated in guineapig but strain died out
bovis	Noguchi (1928)	Seen in film of stomach contents of an ox in the U S A
couvyi	Gomes de Faria (1924)	Seen in blood film of case of dengue fever in Brazil
icterohämoglobinurica	Schuffner (1918)	Seen in blood film of case of blackwater fever in Indonesia
ictero uraemia canis	Klarenbeek (1928)	Seen in urine and kidney sections of dogs suffering from jaundice in the Netherlands
melanogenes canis	Lukes (1924)	Seen in kidney sections of fatal cases of Stuttgart disease in dogs in Brno Czechoslovakia

Leptospires Cultivated in Artificial Media but Lost before Identification (all isolated from man)

Name	*Reference*	*Origin*
Eruthyan	Fletcher (1928)	Malaya
Kebler	Vaucel & Soulier (1937)	French Indo China
Klemens	Fletcher (1928)	Malaya
Nallathamby	Fletcher (1928)	Malaya
Tuyên Quang	Vaucel & Soulier (1937)	French Indo China
vauceli	Collier (1948a)	Name suggested for Tuyên Quang

Leptospires Existing in Culture but not yet Fully Identified (all isolated from man)

Name	Reference	Origin and Relationships	
alexi	Alexander Baer Fair Gochenour King & Yager (195[illegible])	Puerto Rico	
borincano	Alexander Wetmore Evans Jeffries & Gleiser (1955)	Puerto Rico	(Hebdomadis serogroup)
Bafani	van Riel (1946)	Belgian Congo	(Canicola serogroup)
Esposito	Smith & Brown (1955)	Australia	(related to *L. australis A*)
Kabura	van Riel (1946)	Belgian Congo	(Hebdomadis serogroup)
Kambuga	van Riel (1946)	Belgian Congo	(Canicola serogroup)
Kremastos	Smith Brown Tonge Sinnamon MacDonald Ross & Doherty (1954)	Australia	(Hebdomadis serogroup)
malaya	Alexander *et al* (1955)	Malaya	
[illegible]	van Riel (1946)	Belgian Congo	(Icterohaemorrhagiae serogroup)
[illegible]	van Riel (1946)	Belgian Congo	(Icterohaemorrhagiae serogroup)
[illegible]	[illegible]	Australia	(Pyrogenes serogroup)
[illegible]	[illegible]	Australia	(related to *L. grippotyphosa*)

identification of minor antigenic components might prove useful in epidemiological investigations in a manner comparable to the part played by Vi bacteriophages It is possible also, as Fuhner (1950 a) suggested, that minor antigenic differences among strains might account for the variety of 'Paradoxical Reactions' (p 194) sometimes found in the sera of patients during the early stages of illness These however are questions which can only be answered by further observation and experiment

ANTIGENIC SUBTYPES

The stipulation that homologous agglutinins must remain in both antisera after absorption is important because of the existence of so called 'complete' and 'incomplete biotypes' The terms were coined by Gispen and Schuffner (*1939 a*) to describe two antigenic subtypes of *L icterohaemorrhagiae* first recognized by Borg Petersen (1938) He absorbed an anti serum, prepared against strain M20 of *L icterohaemorrhagiae* with strain RGA (the first strain to be grown in culture in Europe) After absorption, the M20 antiserum no longer agglutinated strain RGA but it retained its full titre against its homologous strain, M20 By contrast, no homologous antibodies remained in RGA antiserum after absorption with strain M20

Borg-Petersen concluded that strain M20 possessed antigenic *components additional to those present in strain* RGA There was no indication that strain RGA had originally possessed the additional antigen and had subsequently lost it during prolonged cultivation in artificial media On the contrary, Borg-Petersen found that recently isolated strains, obtained both from rats and from cases of Weil's disease might belong to either subtype There are no differences between the clinical pictures of the disease caused by the two subtypes, so the additional fraction is not apparently associated with virulence

These findings have been confirmed by many other workers In studies of antigenic composition, it is customary to distinguish the two subtypes by designating the 'complete (M20) and incomplete (RGA) biotypes' as *L icterohaemorrhagiae* (AB) and *L icterohaemorrhagiae* (A) respectively

L icterohaemorrhagiae grows on the chorio-allantoic membrane of 10-day-old chick embryos, and this was confirmed by Davi (1939) Brede and Lempfrid (1951) cultured twelve serotype of leptospires in the allantoic fluid of incubated hens' eggs and they believed that this fluid, used instead of rabbit serum improved Korthof's medium

All these media are fluid, or only slightly jellified, and are used in tubes No satisfactory solid medium for surface growth on plates has yet been produced Woratz (1952 reported that he had obtained very slow growth of leptospire on the surface of agar slopes, but he stated later (persona communication) that this belief had been mistaken

GROWTH REQUIREMENTS

Chang (1947 b) reported a long series of experiments with cultures of *L icterohaemorrhagiae* He concluded that leptospires show evidence of living on proteins or amino-acids and of being incapable of using simple carbohydrates (glucose galactose, mannitol, fructose), that they require serum for growth in artificial medium, that the growth factors of serum are destroyed by heating at 100°C for less than one minute and cannot be entirely replaced by a combination of vitamins glycerol, cytochrome-*c* and serum albumin

Chang confirmed that the optimum temperature of incubation is in the range of 25°–30°C and he found, with the small number of serotypes which he used, that *p*H and E_h were not altered during growth (see Frunder, 1952, below) Chang found with his tryptose and liver-extract medium that *L icterohaemorrhagiae* had a generation time of 24 to 48 hours, and showed the greatest density of growth during the third week of culture Fulton and Spooner (1956) found that the mean generation time of the same serotype grown at 25°C in Korthof's medium was 58 to 68 hours during the logarithmic phase and that the peak of growth occurred at about twelve days

By contrast, Faine (1957 b) estimated the generation time *in vivo* of a highly virulent strain of *L icterohaemorrhagiae* to be from 5 8 to 11 6 (average 8 3) hours in guineapigs

The virulence of *L icterohaemorrhagiae* usually decreases progressively in successive subcultures in fluid medium, and Chang (1947 a) believed from a few experiments that the

CHAPTER III

PHYSIOLOGY OF LEPTOSPIRES

Cultural Requirements Chemical Composition, Survival Outside the Animal Body Resistance to Physical and Chemical Agents, including Antibiotics

CULTURAL REQUIREMENTS

Composition of Culture Media

Leptospires are comparatively easily cultivated in artificial medium From the time that Inada *et al* (1916) grew the first strains of *L icterohaemorrhagiae* in a modified form of Noguchi's medium for other spirochaetes (Noguchi, 1912) a naturally-formed body fluid, such as serum, or ascitic or hydrocele fluid, has been the essential constituent of the media used A very simple formula which was recommended by Gardner (1943) is a solution of 12 per cent rabbit serum in glass-distilled water The medium however cannot be recommended for maintaining strains because growth progressively diminishes in serial subcultures

In Fletcher's medium nutrient agar was added, Schüffner used peptone instead of broth, and Sorensen's solution instead of Ringer's He also found that growth was often improved if the rabbit blood was slightly haemolyzed Other recipes such as Korthof's (1932) give precise details of the amounts of peptone, salts, serum and haemoglobin and of their assembly For practical purposes, Korthof's medium is very reliable

Chang (1947 a) elaborated a medium in which tryptose took the place of peptone, and in which liver extract was used in addition to salts, serum, haemoglobin and agar He found that the sera of rabbits, horses or guineapigs were equally satisfactory

Morrow, Syverton, Stiles and Berry (1938) found that

METABOLISM

Using the Warburg apparatus Chang failed to detect any consumption of oxygen and he considered that the need for the presence of oxygen might be explained either by a very small consumption, or by its use as a catalyst However, working with denser suspensions of leptospires Marshall (1949) showed that aerobic respiration could be measured by the Warburg technique Oxygen uptake was markedly stimulated by rabbit serum, but not by carbohydrates, amino acids, haemoglobin or peptone

Fulton and Spooner (1956) made an extensive investigation of the metabolism of *L icterohaemorrhagiae* in Korthof's medium Because of the slow rate of growth and the low metabolic activity of the organisms the authors were not surprised that little chemical change of the medium accompanied growth There was no change in *p*H during 24 hours incubation, glucose was not utilized, and the appearance of minute amounts of some volatile fatty acids was almost the only alteration found in the medium

Concentrated suspensions of the organisms in culture medium consumed oxygen and produced carbon dioxide at a slow but steady rate Serum protein was identified as the active constituent of the medium supporting respiration A cytochrome *c* system was demonstrated by spectroscopy in washed leptospires and was found to be involved in respiration (Fig 9) The respiration of leptospires was not altered by substances like malonate, fluoride and arsenate which inhibit the enzymes concerned with the metabolism of glucose By contrast, respiration was greatly reduced by cyanide and azide, a fact which indicates the presence in the organisms of a cytochrome system

Czekalowski, McLeod and Rodican (1953) confirmed the finding of Dinger (1932) that, in the semi-solid medium, leptospires frequently grow in very narrow zones, or discs, a few millimetres below the surface All the strains they examined produced growth discs, but with pathogenic serotypes the zones were narrower, and occurred farther below the surface, than those which developed in cultures of water leptospires ar' other probably nonpathogenic serotypes From this and

addition of a small amount of an emulsion of fresh liver of healthy young guineapigs maintained the virulence of strains in culture, and even revived it after it had been lost Dinger (1932) found that the leptospires which grew densely in a narrow zone, or disc, in semi-solid medium remained virulent for nearly three years without animal passage

Schneiderman, Greene, Schieler, McClure and Dunn (1951) gave preliminary reports of careful experiments which showed that much of the value of the serum in medium lies in the albumin fraction In general the crystalline, and alcohol precipitated albumins had little or no activity, whereas the fractions precipitated at 71 per cent ammonium sulphate saturation from horse, human and sheep (but not bovine) serum was nearly as effective as dialysed rabbit serum Later the same workers (1953) found that the growth of *L canicola* in a chemically defined medium, lacking amino-acids but supplemented with rabbit serum albumin, was stimulated by certain concentrations of arginine, aspartic acid, glutamic acid or proline Growth was inhibited by any one of 16 amino acids at one or other of the concentrations tested The rabbit albumin could not be replaced by a mixture of amino acids which simulated the amino-acid composition of the albumin as determined by microbiological assay

Thiamine was found to be the only vitamin essential for the growth of *L canicola* *L ballum*, *L canicola*, *L icterohaemorrhagiae*, *L pomona* and *L sejroe* could be maintained in subculture in a simple medium of salts, thiamine, asparagine and rabbit serum albumin Gram and Schlipkoter (1953) showed that growth of leptospires was stimulated by the vitamin T of Goetsch, and to some extent by ten substances of a vitamin nature, mostly of the B complex

It is general experience that a *p*H of the medium of approximately 7 4 is best, and that oxygen is needed for multiplication Chang (1947 b) found that growth of *L icterohaemorrhagiae* was retarded when the E_h of the medium was lowered to 220 millivolts Above this critical level the growth increased steadily, while below it the number of leptospires progressively decreased

Details for the preparation of some of the most dependable media are given in the Appendix

days after the peak period of growth, and thereafter it declined No haemolysis occurred when the mixtures were incubated at 0°C The haemolytic action was greater at 37° than at 30°C, but it was destroyed when the fluid was heated for ten minutes at 56°C before the mixtures were made No reduction in potency was observed either after lyophilization or after storage in the presence of air for four months at +5° and −70°C

The authors suggested that the presence in leptospires of a soluble haemolytic agent may be a significant factor in determining the pathogenicity of leptospiral infections This is a tempting speculation, but the evidence at present available does not appear to favour it For instance, neither of the two strains of *L icterohaemorrhagiae* included in the series formed haemolysin One of these strains had certainly been maintained in culture for many years, and might have lost its ability to form lysins The other strain however was isolated in 1954 By contrast, cultures of three strains of *L pomona*, isolated respectively in 1952, 1953 and 1954, all contained haemolysin

In spontaneous human infections *L icterohaemorrhagiae* causes haemorrhagic lesions much more frequently than does *L pomona* Moreover, the authors stated that the haemolysin had no effect on human, guineapig and hamster erythrocytes Undoubtedly the observations are extremely interesting, and the subject merits further investigation At present however it seems inadvisable to draw any far-reaching conclusions from the facts available

CHEMICAL COMPOSITION

Information regarding the chemical constitution of leptospires is scanty, and such investigations as have been carried out were largely incidental to serological studies Thus Carlinfanti (1938) obtained a lipoid material from several serotypes by alcoholic extraction and the substance appeared to act as a genus specific antigen in complement fixation tests Hiatt (1953) made some investigations of the immuno-chemistry of the Wijnberg strain of *L icterohaemorrhagiae* In his experiments washed leptospires were disintegrated with sodium deoxycholate (0 4 per cent) in the presence of sodium citrate (0 2 M) and sodium chloride (0 3 M) The resulting suspension was shaken

experiments they concluded that leptospires require gaseous oxygen at a tension below atmospheric, that they are cyanide sensitive, have only slight reducing activity, and that they do not produce recognizable amounts of hydrogen peroxide

Very few differences have been found in the cultural requirements or metabolic activities of different serotypes of leptospires One distinction is that saprophytic leptospires, found in water and capable of surviving indefinitely in it in a wide range of conditions, can multiply in a suspension of human faeces (Hindle, 1925), whereas the pathogenic serotypes fail to survive in these conditions Frunder (1952) found that a strain of *L icterohaemorrhagiae* and a strain of *L sejroe* did not change the *p*H of Korthof's medium when the *p*H was 6 9 or 7 4 at the time of inoculation, whereas in the same circumstances *L grippotyphosa* raised the value of the *p*H by a unit

At present, study of the metabolism of leptospires is hindered by their comparatively low rate of multiplication in the known media, and by their inability to grow on the surface of solid medium, from which most other bacteria can be harvested comparatively free from the constituents of the medium

Nothing is known of the possible production by leptospires of toxic substances which might be capable of producing specific skin reactions in infected persons, or of causing the diffuse necrosis of the convoluted tubules of the kidneys which is the most important lesion in leptospiral disease of man and animals However Alexander, Smith, Hiatt and Gleiser (1956) demonstrated the presence of a haemolysin in cultures of some strains of leptospires When erythrocytes from a variety of domestic and laboratory animals were tested, only those from sheep cow and goat proved susceptible to lysis The capacity to elaborate haemolysin was also investigated for a number of strains belonging to a wide range of serotypes Only a proportion of strains possessed the ability to produce the lytic agent, and furthermore different strains of the same serotype sometimes acted in dissimilar ways For example, two strains of *L canicola* produced haemolysin, whereas a third did not

Experiments showed that the lytic agent was present in higher concentrations in the culture fluid, freed from organisms by centrifugation, than in suspensions containing disrupted leptospires The haemolytic titre reached a maximum two or three

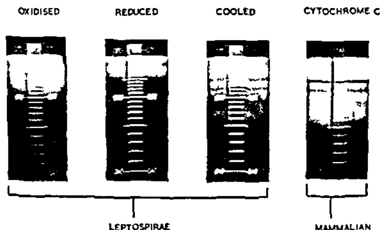


Fig 3

Absorption bands of cytochrome *c* in washed leptospires By Dr J D Fulton and Dr D J Spooner Reproduced from *Experimental Parasitology* by kind permission

with a mixture of amyl alcohol and chloroform and was then centrifuged to separate the phases The aqueous phase was concentrated by pervaporation and dialysed against saline buffered at *p*H 7 The final product (Fraction 1) was a uniform, stable, opalescent suspension containing about 40 per cent of the total solids of the organisms It reacted positively in the Molisch test and negatively in the biuret and ninhydrin tests

A partial analysis gave the following results

	Per cent
Nitrogen	7 0
Phosphorus	1 8
Pentose	8 3
Deoxyribonucleic acid	6 8

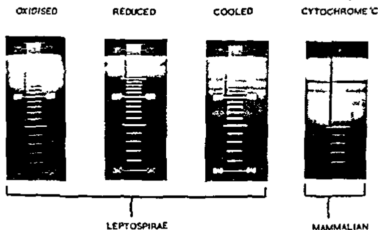
Only about one quarter of the solids were accounted for in the analysis but, taken in conjunction with the qualitative tests, they indicate that the material is free of proteins, peptides and amino-acids The author considered that ribonucleic acid is probably present

Ultraviolet absorption spectra showed a well-defined maxi mum at 258 mμ, a finding which provided confirmatory evidence of the presence of nucleic acids In addition, the characteristic specific absorptivity disappeared after treatment with ribonuclease and deoxyribonuclease, followed by dialysis

Fraction 1 contained a component which fixed complement in the presence of homologous and to a lesser extent of hetero logous immune sera The activity was not diminished after treatment with the enzymes mentioned above, nor with hemi cellulase

When three volumes of ethanol were added to one volume of Fraction 1 a fibrous precipitate formed which contained most of the nucleic acid The supernatant fluid, after removal of the alcohol by dialysis retained at least 80 per cent of its original activity The dried residue was insoluble in diluted saline absolute ethanol and ether Its activity was not reduced by thorough extraction with boiling ethanol

The reactive substance was thus neither protein not lipid, and was probably a polysaccharide, predominantly pentosan in structure *A partial analysis gave the following results which* are consistent with that supposition



F g 1

Absorpt on bands of cytochrome c in washed leptospires By Dr J D Fulton and Dr D F Spooner Reproduced from Experimental Paras tology by k nd perm ssion

	Per cent
Carbon	44 88
Hydrogen	6 89
Nitrogen	6 9
Phosphorus	1 5
Sulphur	0 18
Ash	11 43
Pentose	19 4
Deoxyhexose	14 5

When injected into rabbits Fraction 1 stimulated the production of specific complement-fixing antibodies, but the antiserum did not agglutinate living homologous leptospires It appears therefore that this active principle is not identical with the specific agglutinogens of the serotype but represents an independent antigenic system

Hiatt's findings on the chemical constitution of Fraction 1 were confirmed by Schneider (1953) In a later study Schneider (1954 a) used the same deoxycholate method of obtaining the active material from *L bataviae* and from *L canicola* also Chemical analyses showed that Fraction 1 contained about 1 per cent organic phosphorus, and from 3 to 9 per cent organic nitrogen, together with deoxypentosenucleic acid and complex polysaccharide material Pentoses and methylpentose were among the carbohydrate residues of the polysaccharide portion Chromatography of hydrolysates of Fraction 1 of *L ictero-haemorrhagiae* revealed the presence of five to seven components, some of which migrated at rates similar to those of reference standards of l(—) arabinose, d(—) xylose, l(—) rhamnose and glucosamine

A preliminary identification of certain of the polysaccharides in Fraction 1 was made by means of the sulphuric acid-sulphydryl colour (B Cy R) reaction of Dische (1949) The ultraviolet absorption spectra are shown in Fig 10 in which Curve I is in each instance, the reaction mixture without cysteine Curves II are of sugars to which cysteine was added and measurement made 15 to 45 minutes later (primary reaction) Curves III were obtained from the same mixtures after [illegible]

re

of

present in the samples were calculated from data obtained from mixtures of these carbohydrates which were included in the test for comparison The spectra indicated the presence of similar or identical monosaccharide residues in the preparations derived from the different serotypes These analytical procedures do not therefore provide any information regarding the factors responsible for serological differences among serotypes

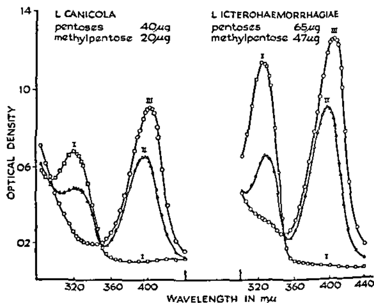


Fig 10

Ultraviolet absorption spectra of Fraction 1 antigens in Dische s sulphuric acid sulphydryl (BCyR) reaction mixture By Dr M D Schneider Reproduced from Journal of Infectious Diseases by kind permission

In a later communication, Schneider (1955) reported on the antigenic qualities of Fraction 1 He found, as did Hiatt, that rabbits injected with the substance produced complement-fixing antibodies which were active in the presence of all the heterologous leptospiral antisera tested By contrast however Schneider could also demonstrate agglutinins in the Fraction 1 antisera and these agglutinins proved to be sharply serotype-specific

Further fractions were prepared by Schneider (1954 b)

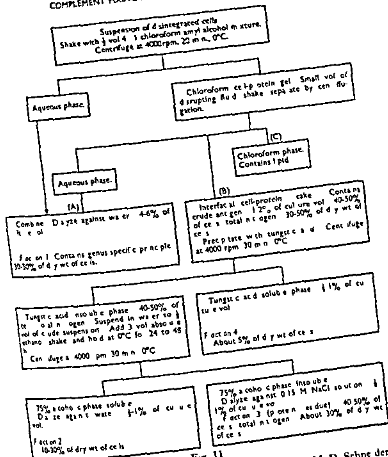


Fig 11

Scheme for fractionation of washed viable leptosp rea By Dr M D Schne der Reproduced from Proceedings of the Society for Experimental B ology and Med cine by kind perm ssion

according to the scheme shown in Fig 11 The numerical sequence—Fractions 1, 2 3 and 4—represents decreasing amounts of serologically active material in the fractions and not the order in which they were isolated

The chemical composition of Fraction 2 differed from that of Fraction 1 by its lower content of nitrogen pentose and

deoxyhexose, by the apparent absence of glucosamine, and by the presence of an unidentified ultraviolet absorption peak at 385 to 390 mμ The cysteine sulphuric acid colour complexes (Dische and Shettles, 1948) have an absorption band located at 265 to 275 mμ, and a very sharp peak at 385 to 390 mμ. On a weight basis the extinction co efficient at 388 mμ was greatest for *L canicola*, less for *L icterohaemorrhagiae* and least for *L bataviae*

Fraction 3 was predominantly protein in nature, and contained about 40 per cent of the total nitrogen of the cell Fraction 4 consisted of non-protein nitrogen material Both these fractions fixed complement in low degree, possibly because they were contaminated with carbohydrate residues from Fraction 1

In a further study of Fraction 1 of *L canicola* and Fractions 1, 2 and 3 of *L bataviae* and *L icterohaemorrhagiae*, Schneider and McLaughlin (1955) used infrared spectrophotometry, by means of which certain chemical functional groups can be distinguished In the range from 2 to 15 μ used in this investigation proteins show strong bands at 3 0, 6 1 and 6 5 μ Fraction 3 of both serotypes, which contains 40 to 50 per cent of the cell nitrogen, shows peaks at these wave lengths This was to be expected as the previous analyses had shown this fraction to be predominantly protein in nature The similarities in the absorption spectra of Fraction 3 materials prepared from *L bataviae* and *L icterohaemorrhagiae* suggest that the protein constituents are common to both (Fig 12)

Fraction 1 material from all three serotypes showed a band which represents a group of absorbances between 9 0 and 9 75 μ This band appears to be related to polysaccharide linkages (Whistler and House, 1953) Nucleic acid is considered to be responsible for a band at 8 0 to 8 1 μ, and this would seem to be consistent with the chemical data, since deoxypentose-nucleic acid comprised about 8 per cent of the density of Fraction 1 This band was absent in Fractions 2 and 3 The band at 3 0 μ is a composite of NH, OH and CH stretching The absorption at 3 45 μ has been attributed to CH stretching, and that at 6 25 μ to a carboxylate structure and zwitterion type

Fraction 2 shows marked absorption at 3 45 μ and a carboxyl peak, absent in Fraction 1, occurs from 5 7 to 5 8 μ The peaks from 6 0 to 6 75 μ are more numerous, but suggest basic

similarities to Fraction 1 The band from 9 0 to 9 75 μ shows several smaller peaks, indicating that a more complex saccharide may be present in Fraction 2

When the spectra of Fraction 1 and Fraction 2 of the serotypes are compared in detail they exhibit significantly different absorption bands, the full interpretation of which is not yet

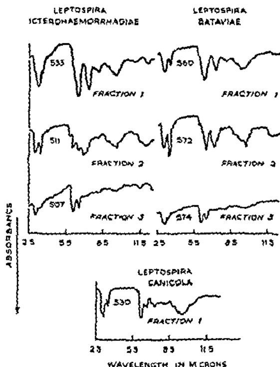


Fig 12

Infrared absorption spectra of fractions of two leptospires By Dr M D Schneider and Dr J McLaughlin Reproduced from Journal of Bacteriology by kind permission

possible The authors considered however that an extension of the study of the spectral bands of the polysaccharide-containing constituents of leptospires might throw fresh light on the causes of serological differences and relationships

Rothstein and Hiatt (1956) found that a 70 per cent ethanol

extract of leptospiral bodies contained both complement-fixing and precipitinogen antigens On prolonged electro-dialysis the former was precipitated and the precipitinogen remained in solution Chemical analysis showed the presence in both antigens of pentose and methylpentose, and the absence of hexose The two antigens also had similar infrared absorption spectra

When injected into rabbits the precipitinogen stimulated the production of two types of antibody —(1) precipitins which reacted with the precipitinogens from a wide variety of serotypes, and (2) agglutinins which were serotype-specific The authors concluded that two antigenic components must be present One, the P-antigen, situated on the periphery of the cell normally acts as an agglutinogen, but can act in certain circumstances as a precipitinogen and complement-fixing antigen The other, S antigen, is somatic, it plays no part in reactions involving intact leptospires, but would be exposed when the cell is disrupted

SURVIVAL OUTSIDE THE ANIMAL BODY

Survival in Water

The time of survival of leptospires under different adverse physical and chemical conditions has been only ' studied. In particular, possible differences ' genic serotypes in this respect have not l The data so far recorded have *haemorrhagiae* and, less f... leptospires As would be ... water leptospires survive for domestic water supplies, as from tap water in London by by Schlipkoter (1951) The specimens of tap water, taps which had not been richer in leptospires than after the water had been

These findings are in nonpathogenic water ' water, while the path

tissues of animals Outside the body pathogenic serotypes grow very little, and they tend to die at a rate varying with conditions This supposed difference between the nonpathogenic and the pathogenic serotypes cannot be precisely confirmed by recorded observations, and the position of the pathogenic serotypes stated above is not wholly accepted by van Riel (1948) He believes that animals are of secondary importance as reservoir hosts, and that water is the more fundamental habitat

FRESH WATER Noguchi (1918 b) added *L icterohaemorrhagiae* to distilled water and noted that the organisms all disappeared within 7 days On the other hand, van Thiel (1937) infected a sample of surface-water with a virulent strain of *L icterohaemorrhagiae*, and was able to prove the continued presence of leptospires for at least 22 days by means of the '*guineapig bathing test*' (see Appendix) He noted that the leptospires tended to sink to the lower levels, and that they could best be detected by stirring up the water before taking samples for test

Chang, Buckingham and Taylor (1948) found that *L icterohaemorrhagiae* remained viable for 18 to 20 days in tap water which was allowed to become contaminated with bacteria from the air In water of *p*H 7 0 from the Charles River, Boston, Mass, the survival time was 5 days In sterile water the leptospires survived for 28 hours at *p*H 5 0, and for 30 days at *p*H 7 0 When 1 per cent of serum or 0 1 per cent tryptose was added, the survival times were 100 and 50 days respectively

SALT WATER The deleterious effect of sodium chloride on leptospires is shown by the lesser risk of contracting infection when bathing in salt water as compared with fresh Ruys (quoted by Schuffner, 1934) tested the survival of *L icterohaemorrhagiae* in water from various sources in the Netherlands and from the North Sea on the Dutch coast She found that in lake water with a low salinity, 40 mg of chlorine per litre or less, leptospires survived for 10 days, whereas in water from the North Sea with 13,000 to 17,000 mg the survival was less than 1 day Broadly speaking, in waters of intervening values leptospires survived for proportional times Similarly, Chang *et al* (1948) recorded that in sea water with a total salt content

of 22,000 mg per litre, *L icterohaemorrhagiae* survived for 18 to 20 hours

Ruys (1946) reported that, during the summer of 1944, there were no instances in Amsterdam of Weil's disease due to bathing or sudden immersion She attributed this to the fact that during those months the canals were flooded with sea water, so that in one particular canal the salinity rose in August to 1,572 mg of chlorine per litre, contrasted with 639 to 1,045 mg in other years

The use of sea water for washing fish was considered by Hampson (1946) as a likely explanation of the low incidence of Weil's disease among fish workers in Grimsby, England, as compared with the high rate in Aberdeen, Scotland, where fresh water was used (Davidson, Campbell, Rae and Smith, 1934) In like manner Goudie, Weir and Wilson (1952) attributed some of the freedom from Weil's disease of fish workers in the Glasgow fish market to the fact that the fish curers there immerse their hands from time to time in a solution of synthetic dyestuff containing 25 per cent of common salt

Contaminated Water and Sewage *Noguchi (1918 b) had* also observed that leptospires survived only for a few days in badly polluted waters This finding was confirmed by Chang *et al* (1948) who reported that in domestic sewage of *p*H 6 9 the survival time was only 12 hours On the other hand Alston (1935) isolated *L icterohaemorrhagiae* from the floor of a sewer and from the outlet of a drain in a sewer The same author (1948) found that leptospires retained their virulence for at least 24 hours in sewer mud

Survival in Milk

Kirschner, Miller and Garlick (1952) showed that leptospires survived only a short time in undiluted milk, but for at least two months in milk diluted 1/20 with rain or tap water Kirschner and Maguire (1955) continued this work They found that virulent and nonvirulent strains of [illegible] *icterohaemorrhagiae* and *L pomona* were lysed [illegible] cows
goats and human beings [illegible] by [illegible] m)
dilutions varying from [illegible] 0 [illegible]
effect was not reduced [illegible] ge [illegible] 4°C
for two months, or by [illegible] 2

hours It withstood pasteurization and heating for 5 minutes at 80°C but was destroyed by boiling The active agent was neither lysozyme nor lactenin The authors concluded that this agent accounts for the absence of milk borne infections in the many countries where bovine leptospirosis occurs

SURVIVAL IN URINE

It has long been recognized that leptospires die rapidly after excretion in the urine of patients Davidson and Smith (1936) added a few drops of culture of *L icterohaemorrhagiae* to samples of urine obtained from ten patients who were not suffering from Weil s disease Drops of the mixtures were examined microscopically at intervals Actively motile lepto spires were seen in all the specimens after 1 hour in 4 after 2 hours and in 3 after 6 hours

It is generally considered that the highly acid reaction of the urine caused by the illness is responsible for this There fore when attempts are made to isolate leptospires by culture or animal inoculation the urine should either be rendered alkaline immediately it is passed or the patient given alkalies to alter the reaction *in vivo*

There must however be other factors involved also because Fuhner (1950 b) observed a similar effect in rat urine even when it was neutral or slightly alkaline in reaction He made microscopical examinations of the urine of carrier rats at various intervals after it was passed The urine contained large numbers of leptospires when it was shed but few active ones were visible after 5 hours and none at all after 24 hours The organ sms did however survive for longer periods in diluted urine

Davidson and Smith also observed lytic antibodies in the urine in some cases van der Hoeden (1936) demonstrated both specific agglutinins and lysins in the urine of men dogs and wild rats as the result of infection In one man they could still be detected more than 3 years after recovery Nowicki (19₅5) investigated the development of antileptospiral lysins in the urine of rabbits injected with *L pyrogenes* and found evidence that the lysins in the urine could be detected earlier than agglutinins in the blood serum From observations on two patients suffering from Weil s disease he suggested that

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with the surface layer of the soil in each of 3 jars After 8 days, enough rain water was added to the first jar to saturate the soil and leave free water on the surface The second and third jars were similarly treated after 15 and 43 days respectively Samples of water were tested for the presence of leptospires by allowing the water to flow through a tunnel in the subcutaneous tissue of guineapigs (see Appendix) By this means, samples from the jar flooded at 8 days were found to be infective after a further 3, 17 and 24 days From the 15-day jar they were infective after another 3 and 10 days, and from the 43 day jar after 3 days, but not after 10 and 17 days

In further experiments the soil was contaminated by keeping, on racks above it, rats known to be excreting large numbers of *L australis A* The rats were removed after 3 to 6 days, and the jars were flooded 8, 15, 22 and 28 days later, respectively Samples from the 8-day jar were infective after 3, but not after 66 days, from the 15-day jar, after 4 days, but no later samples were tested Samples taken after 3 days from the 22-day and 28-day jars proved noninfective

In this connection it is interesting to note that Sawers (1938) stated that the heat generated by burning the trash of sugar canes (carried out before cutting is begun) did not appreciably penetrate into the soil, although it would be sufficient to destroy leptospires on the cane and on the surface of the ground

The effect of the moisture content of soil was studied by Okazaki and Ringen (1957) in sets of tubes containing 2 g of sterilized soil One set was allowed to dry, to the second, sterile distilled water was added to the point of dampness, and sufficient water was added to the third to a point beyond saturation where water covered the soil Each tube was inoculated with about 10 million *L pomona* and kept at room temperature, water was added to the tubes of the second and third sets as necessary to maintain them at approximately a constant moisture content At intervals the soil in one tube of each set was suspended in 10 ml of water The suspension was examined microscopically for the presence of motile leptospires, and was also inoculated into culture media

In dry soil no leptospires could be detected after half an hour or by culture after 2¼ hours In damp soil they could not be seen after 3 days nor cultured after 5 days Under

the appearance of lysins in the urine might be a valuable early indication of infection These lysins must differ essentially from those associated with agglutinins, because antibodies appear in the blood at an earlier stage than in the urine (Davidson and Smith, 1936) The subject therefore needs further investigation

Mason (1938) found that 6 out of 11 rat catchers had antibodies to *L icterohaemorrhagiae* in the blood although they gave no history of previous jaundice In view of van der Hoeden's finding, these men who are liable to repeated subclinical infections might have agglutinins and lysins in the urine also In this connection is it interesting to note that Brown and Cleveland (1932) reported that it was a common practice with some rat catchers to urinate immediately on the wound of a ferret which had been bitten by a rat, because they believed that this was the way to prevent the ferret from dying

Professional rat catchers might be expected to provide several examples of Weil's disease, but we know of only two in Great Britain It may be that experienced rat catchers urinate not only on the bites of their ferrets, but on their own bites as well A rat catcher known to one of us may have further increased his immunity by his occupational side-line, which was biting off the heads of rats outside taverns for a small reward

Survival in Infected Tissue

Mantovani (1950 b) infected pieces of diaphragm of cattle with *L icterohaemorrhagiae* obtained from tissues and urine of guineapigs which had died of the infection Pathogenicity tests on guineapigs, after the tissues had been kept for 3 days at a temperature between +2 8°C and −2 8°C, showed that the leptospires had survived and were still virulent

Survival in Soil

Smith and Self (1955) made careful experiments on the survival of *L australis A* in soil They placed in glass jars blocks of soil about 10 cm in thickness, and added to them a little rain water to moisten the soil thoroughly, the *p*H of the soil was 6 1 to 6 2 In the first experiments, 20 ml of a culture of a recently isolated strain of *L australis A* was mixed

present in waters of pH 6 9 or more, but were absent from those of pH 6 6 or less

Okazaki and Ringen (1957) noted that the time of survival of *L pomona* in solutions of different acidities varied with the temperatures at which they were maintained Thus at pH 6 2 the survival time was longer at 7° to 10°C than at 20° to 26°C whereas at pH 8 4 the reverse effect obtained At −20°C no differences were observed at the different pH levels suggesting that death was due to the temperature alone

DISINFECTANTS

Chang *et al* (1948) reported that elemental iodine destroyed all the leptospires (in water containing 1 to 3 million organisms per ml) in 1 minute when the residual iodine was 5 parts per million, and in 10 minutes when the concentration was 0 7 p p m They found also that Halazone under nearly the same conditions killed all leptospires in 1 minute when the residual chlorine was 3 5 p p m At pH 5, calcium hypochlorite killed all leptospires in 1 and 3 minutes when the chlorine residues were 0 5 and 0 3 p p m respectively, at pH 8 it acted similarly in 1 and 3 minutes with residues of 6 and 3 p p m

They also stated that the cationic detergents Ceepryn Fixanol and Sapamine killed all leptospires in 5 10 30 and 60 minutes at average doses of 30 20, 10 and 7 p p m respectively, but that two anionic detergents required dosages of 1 000 p p m or more Davidson and Smith (1936) had previously shown that sodium hypochlorite in a concentration of 1/4 000 killed *L icterohaemorrhagiae* in 5 minutes

ANTIBIOTICS

Most of the antibiotic drugs have been tested for antileptospiral activity *in vitro* and *in vivo* Several workers found that penicillin inhibited the growth of *L icterohaemorrhagiae* in suitable culture media when the drug was present in concentrations of 0 1 to 0 4 Oxford units (0 06 to 0 24 μg) per ml or more (Alston and Broom, 1944, Chang 1946 Schlipkoter and Beckers 1951) Chang considered that the action was only bacteriostatic, but Alston and Broom believed there was a marked bactericidal action as well Penicillin was found to have a similar degree of inhibitory effect on other serotypes, including *L canicola*, *L*

supersaturated conditions they were still visible for 193 days, and could be cultured up to 183 days

RESISTANCE TO PHYSICAL AND CHEMICAL AGENTS

Heat

A careful study of the thermal death points of leptospires was made by Chang *et al* (1948) They found that, when suspended in water, *L icterohaemorrhagiae* was killed by exposure to a temperature of 45°C for 30 minutes, of 50°C for 10 minutes, of 60°C for 10 seconds, at 70°C the organisms survived for less than 10 seconds

Other Physical Agents

Leptospires will not withstand long exposure to direct sunlight, and it is usually stated that they cannot survive dehydration However, Annear (1956) evolved a technique of preserving the organisms in the dry state for at least 2 years He prepared sterile plugs by freeze-drying 0 25 ml amounts of a solution containing 6 per cent Evans peptone and 0 5 per cent starch Each plug was inoculated with a single drop of culture, concentrated to one twentieth of its original bulk, and the tubes were immediately exposed over P_2O_5 to a high vacuum

Polanen (1941) showed also that they were destroyed by ultraviolet light This finding was confirmed by Alston (1948) who found that, in sewer mud, virulent *L icterohaemorrhagiae* survived exposure to an ultraviolet lamp for 5 but not for 10 minutes

Acids

Leptospires are very sensitive to the action of acids, and they are seldom found in waters of reaction less than *pH* 6 8 For instance, Sardjito and Zuelzer (1929) found them to be abundant in the alkaline waters of Sumatra, but practically absent from the more acid waters of Java As a result, human leptospirosis is common in the former island and comparatively rare in the latter Similarly, in the Andaman Islands, Taylor and Goyle (1931) recorded that leptospires were frequently

by 1 4 or 0 7 μg per ml Oxytetracycline (terramycin) inhibited *L autumnalis* at 2 0 μg, *L canicola* at 0 5 μg, and the other serotypes at 0 2 μg per ml All serotypes showed considerable resistance to chloramphenicol, especially *L autumnalis* which was unaffected by 14 μg per ml In similar experiments with other chemical compounds mepacrine (atebrin) inhibited all the serotypes at a concentration of 10 μg per ml, but no effect was obtained with supranol, plasmoquine, resochin and quinine in concentrations up to 20 μg per ml

Fulton and Spooner (1956) showed that chlortetracyline and oxytetracycline markedly depressed the oxygen uptake of cultures of *L icterohaemorrhagiae* while penicillin and streptomycin had no effect

Ormsbee (1953) found that erythromycin had an inhibitory effect when it was injected into the yolk sac of embryonated hen eggs previously inoculated with *L icterohaemorrhagiae* Faine and Kaipainen (1955) tested the sensitivity of several serotypes to erythromycin in ten fold serial dilutions in culture medium The concentrations of the drug which sterilized the cultures after incubation at 30°C for 10 days were —

Sensitive to 0 001 μg per ml —

L canicola (strain Utrecht IV),
L grippotyphosa L hyos, L sejroe

Sensitive to 0 001 to 0 01 μg per ml —

L icterohaemorrhagiae,
L canicola (strain Aldgate)
L australis A, L bataviae,
L hebdomadis

Sensitive to 0 01 μg per ml —

L pomona

Katsura and Yoshida (1957) found that Tropolon and Hinokitiol (m-isopropyltropolon) had a marked inhibitory and lethal effect on the growth of leptospires *in vitro* The leptospirocidal concentrations were of the same order as those of a number of antibiotics when the drugs were added to the medium before it was seeded with leptospires The Tropolons still had a lethal effect, in relatively low concentrations, when

they were added to cultures which were in the logarithmic and static phases of growth Streptomycin had a similar but much slower effect in the later stages of growth, the other antibiotics (including penicillin, chlortetracycline and oxytetracycline) were practically inactive even in concentrations forty times greater than those which were lethal in the lag phase

From all these observations and experiments on the ability of leptospires to withstand the deleterious action of physical and chemical agents, it appears that they have a comparatively low resistance as compared with many other pathogenic micro-organisms



I k. 13

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Fig 14

Chronic nephritis in a dog due to infection by *L. canicola*, showing patchy fibrosis (× 45)

CHAPTER IV

RESERVOIR HOSTS AND DISTRIBUTION OF LEPTOSPIRES

Host of Election, Intensity of Infection
Mode of Transmission

RESERVOIR HOSTS

The pathogenic leptospires are primarily parasites of animals, more especially rodents, although some serotypes are most commonly found in dogs, pigs or cattle. Leptospires will grow in developing hen eggs and can cause experimental infections in young chicks (p. 184) but no spontaneous infections in birds have been recorded. van Thiel (1946, 1948 b) failed to infect fish and frogs experimentally with *L. icterohaemorrhagiae*. The possibility of more extensive infection of wild rodents by leptospires is illustrated by the work of Packchanian (1940) who found that 26 of the 32 species and subspecies of rodents he tested were susceptible to infection by *L. icterohaemorrhagiae* although they were not known as natural carriers of the organism. In many instances the leptospires appear to have become so well adapted to their hosts that they produce large colonies in the convoluted tubules of the kidneys (Fig. 13) without, as a rule, causing any deleterious effect on the animal. Leptospires are shed in the urine, sometimes in very large numbers.

The length of time during which the carrier state persists is not accurately known, and may perhaps vary according to serotype and host. Fuhner (1950 b) found that spontaneously infected wild rats were still excreting *L. icterohaemorrhagiae* after 8 months in captivity. Walch-Sorgdrager (1939) kept wild rats under observation for as long as two and a half years, during which time they regularly passed leptospires, though in varying numbers. McIntyre and Montgomery (1952) were able to demonstrate *L. canicola* in the kidneys of dogs at least 3 years after recovery from the naturally acc[illegible]

TABLE IV—*continued*

CARRIER HOSTS AND ANIMALS SUSCEPTIBLE TO INFECTION WITH LEPTOSPIRAL SEROTYPES

Serotype	*Pre eminent Carrier Hosts*	*Carriers of Subsidiary or Unknown Importance*	*Other Animals Known, or Considered on Serological Evidence (S), to be Spontaneously Infected*
medanensis	Unknown	Dog	Goat (S), Opossum (S), E spelaea (S), R mulleri (S), R sabanus (S)
wolffii	*Unknown*	—	E *spelaea* (S), R whiteheadi (S)
hardjo	Unknown	—	—
sejroe	Ap sylvaticus, Mu *musculus sp*	Mr agrestis, Mr arvalis	Cattle (S), Dog (S), Horse (S), Pig (S)
saxkoebing	Ap flavicollis, Ap sylvaticus	Mr agrestis, Mu musculus	Buffalo (S), Cat (S), Cattle (S), Dog (S), Goat (S), Horse (S), Pig (S), Cl glareolus (S) R exulans (S), R sabanus (S), Su murinus (S)
bataviae	Mm minutus soricinus, R norvegicus	Mr agrestis, Mr arvalis, R sabanus	Cat (S), Cattle (S) Dog (S), Pig (S), R argentiventer (S), R mülleri (S)
paidjan	Unknown	—	—
semarang	R brevicaudatus	—	—
andaman A	Unknown	—	—
hyos	*Pigs*	R *bowersi*	*Cattle* (S), *Horse* (S)
celledoni	Unknown	—	—
mini	Unknown	—	—

the other hand Popp (1950) brought forward evidence (p 153) which suggested that *Microtus arvalis* may act as a carrier of *L grippotyphosa* for only a few months

In other instances the adaptation is less complete Thus dogs infected with *L canicola* develop chronic interstitial nephritis, and progressive fibrosis eventually destroys much of the kidney tissue (Fig 14) According to Popp (1950) *L grippotyphosa* produced acute nephritis in *Microtus arvalis* and this led to the death of a considerable proportion of the carriers

Host of Election

Broadly speaking, each leptospiral serotype tends to be associated with a particular species of carrier host for example *L icterohaemorrhagiae* with *Rattus norvegicus* and *L canicola* with dogs There are however many exceptions to this rule, because one serotype may be carried by different hosts, and one species of animal may act as host to different serotypes Thus *L bataviae* is usually carried by *R norvegicus* in Indonesia and by *Micromys minutus soricinus* in Northern Italy Conversely, pigs may act as hosts to *L canicola*, *L hyos* and *L pomona* In Tab

hosts

believe

noted that no reservoir hosts have yet been identified for a number of serotypes, such as *L andaman A*, *L celledoni*, *L mankarso* and *L naam*

Intensity of Infection —Within a population of animals which are potential carriers the proportion actually infected may vary greatly even in areas close to one another During one outbreak of field fever the infection rate with *L grippotyphosa* among *Microtus arvalis* caught in adjacent localities ranged from 5 to 80 per cent Comparable, though less widely divergent variations were recorded for infections with *L icterohaemorrhagiae* among *R norvegicus* in a rural area in South Wales (Broom and Gibson, 1953)

A high density of rodent population probably tends to raise the infection rate among them because of the increased chance of individuals coming in contact with infective urine Other factors however appear to be involved also Seasonal fluctuations in carrier rates among rats has been suspected by

The number of *antigenically distinct* serotypes present in different localities also varies widely About a score have been identified in Indonesia (Collier, 1948 a) and in Malaya (Gordon Smith and Broom, to be published) In Australia, 13 separate serotypes have been isolated and identified (Queensland, 1955) and 8 in the U S A (Yager, 1953, Klatskin, 1955)

According to Rimpau (1952) the presence of 15 serotypes has been established in Europe His list includes however certain serotypes *isolated and named in the U S S R which* Kmety (1955 b) showed to be identical with serotypes already known Therefore the exact total for Europe is uncertain but is probably about 12

The dissemination of these serotypes throughout the different countries in Europe also shows considerable variations Gsell (1952) has recorded 8 from Switzerland and also from Northern Italy, Borg-Petersen (1949) 7 from Denmark, and Salminen (1956) 7 from Finland By contrast only 2 serotypes, *L icterohaemorrhagiae* and *L canicola,* have been found in Great Britain

These disparities may be the result of a number of factors In some countries, such as the U S A (Larson, C L, 1953) and Malaya (Broom, 1953 a), leptospirosis was regarded as of little importance until the intensive work carried out within recent years showed this view to be greatly mistaken It is possible therefore that new endemic areas may be discovered as the result of future investigations In other instances the explanation may lie in differences in environmental conditions Thus *Sardjito and Zuelzer (1929) found a much higher incidence of* leptospirosis in Sumatra, where the water tended to be slightly alkaline in reaction, than in Java where the reaction was more acid

The absence from Britain of serotypes other than *L icterohaemorrhagiae* and *L canicola* is not due to the lack of the appropriate reservoir hosts As Alston (1949) pointed out, the species of Muridae which on the Continent carry *L ballum, L bataviae, L grippotyphosa* and *L sejroe* are present in the British Isles, and an additional serotype, *L saxkoebing,* occurs in rodents nearly related to British species (Table VI)

Comments and hypotheses on the distribution of various serotypes throughout the world were made by Broom (1953 b)

He contrasted the almost world-wide distribution of serotypes such as *L. icterohaemorrhagiae* with others like *L. andaman A* which appears to be confined almost entirely to the Andaman Islands in the Indian Ocean. In seeking an explanation for this disparity, he speculated about the means by which a single serotype spreads across the world and about the factors which influence the emergence of new serotypes.

TABLE VI

MURIDAE (EXCLUDING RATS) WHICH CARRY LEPTOSPIRES ON THE CONTINENT OF EUROPE BUT NOT IN GREAT BRITAIN

(After Alston 1949)

British Muridae	*Continental Muridae*	*Serotypes Carried*
MICROTINAE (voles and lemmings)		
Clethrionomys glareolus	*Clethrionomys glareolus*	*L. grippotyphosa*
Microtus agrestis	*Microtus agrestis*	*L. grippotyphosa*
Microtus hirtus		
	Microtus arvalis	*L. grippotyphosa*
Arvicola amphibius	*Arvicola amphibius*	
	Arvicola sherman	*L. icterohaemorrhagiae*
MURINAE (excluding rats)		
Apodemus sylvaticus	*Apodemus sylvaticus*	*L. grippotyphosa*
		L. sejroe
		L. bataviae
Apodemus flavicollis	*Apodemus flavicollis*	*L. saxkoebing*
Micromys minutus	*Micromys minutus*	*L. bataviae*
Mus musculus	*Mus musculus*	*L. ballum*
		L. saxkoebing
		L. sejroe

The westward spread of *L. icterohaemorrhagiae* in Eurasia, for example, may have been the result of the migration of *R. norvegicus*, which is the principal carrier host of that serotype. It is believed that up to 1720 *R. norvegicus* had not penetrated further west than the River Volga in Russia. Following a few years of plenty during which the rat population increased enormously, a bad season precipitated a vast westward migration across that river. The Baltic ports were invaded about 1729 and thence the brown rat was carried in ships to various countries, including England. If we assume that in those days *R. norvegicus* was infected with *L. ictero-*

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
ASIA		ASIA	
Indonesia	*L australis A*	Malaya—*contd*	*L bangkinang*
	L autumnalis		*L bataviae*
	**L bangkinang*		*L benjamin*
	**L bataviae*		*L canicola*
	**L benjamin*		*L celledoni*
	L canicola		*L djasiman*
	**L cynopteri*		*L grippotyphosa*
	**L djasiman*		*L hebdomadis*
	L grippotyphosa		*L hyos*
	**L hardjo*		*L icterohaemorrhagiae*
	L hebdomadis		*L javanica*
	L icterohaemorrhagiae		*L medanensis*
	**L javanica*		*L naam*
	**L mankarso*		*L poi*
	**L medanensis*		*L pomona*
	**L naam*		*L pyrogenes*
	**L paidjan*		*L schuffneri*
	L pomona		*L semarang*
	**L pyrogenes*		*L sentot*
	**L sarmin*		*L wolffi*
	**L schuffneri*		
	L sejroe	Pacific Islands	
	**L semarang*	Hawaii	*L canicola*
	**L sentot*		*L icterohaemorrhagiae*
	**L wolffi*		
		Okinawa	*L autumnalis*
Iraq	*L grippotyphosa*		*L hebdomadis*
Israel	*L canicola*	Samoa	*L australis A*
	L grippotyphosa		*L icterohaemorrhagiae*
	L pomona		
		Thailand	*L autumnalis*
Japan	*L australis A*		*L bataviae*
	**L autumnalis*		
	L bataviae		
	L canicola		
	**L hebdomadis*		
	**L icterohaemorrhagiae*	AFRICA	
	L pyrogenes	Algeria	*L icterohaemorrhagiae*
Korea	*L icterohaemorrhagiae*	†Belgian Congo	*L australis A*
			L bataviae
Lebanon	*L grippotyphosa*		*L benjamin*
	L icterohaemorrhagiae		*L canicola*
			L grippotyphosa
†Malaya	*L australis A*		*L hyos*
	L australis B		*L icterohaemorrhagiae*
	L autumnalis		*L pomona*
	L ballum		*L wolffi*

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA Egypt	*L grippotyphosa* *L icterohaemorrhagiae* *L sejroe*	AMERICAS West Indies—*continued* Trinidad	*L icterohaemorrhagiae*
Kenya	*L grippotyphosa*	South America Argentina	*L canicola* *L hyos* *L icterohaemorrhagiae* *L pomona*
French Morocco	*L icterohaemorrhagiae*		
French West Africa	*L icterohaemorrhagiae*	Brazil	*L australis B* *L canicola* *L icterohaemorrhagiae*
Madagascar	*L grippotyphosa* *L pomona*	Chile	*L canicola* *L icterohaemorrhagiae* *L pomona*
Tunisia	*L grippotyphosa* *L icterohaemorrhagiae*	Ecuador	*L icterohaemorrhagiae*
AMERICAS North America Canada	*L ballum* *L icterohaemorrhagiae* *L pomona*	French Guiana	*L icterohaemorrhagiae*
Mexico	*L icterohaemorrhagiae*	AUSTRALASIA †Australia	**L australis A* **L australis B* *L canicola* **L celledoni* **L hyos* *L icterohaemorrhagiae* *L medanensis* **L mini* **L pomona*
U.S.A.	*L autumnalis* *L ballum* *L bataviae* *L canicola* *L grippotyphosa* *L icterohaemorrhagiae* *L pomona* *L sejroe*		
West Indies Cuba	*L canicola* *L grippotyphosa* *L icterohaemorrhagiae*	New Zealand	*L canicola* *L icterohaemorrhagiae* *L pomona*
Guadeloupe	*L icterohaemorrhagiae*	Papua and New Guinea	*L andaman A* *L bataviae* *L hyos* Autumnalis serogroup Hebdomadis serogrou Pyrogenes serogroup
†Puerto Rico	*L ballum* *L bataviae* *L canicola* *L icterohaemorrhagiae*		

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*
ASIA	
Indonesia	*L australis A*
	L autumnalis
	**L bangkinang*
	**L bataviae*
	**L benjamin*
	L canicola
	**L cynopteri*
	**L djasiman*
	L grippotyphosa
	**L hardjo*
	L hebdomadis
	L icterohaemorrhagiae
	**L javanica*
	**L mankarso*
	**L medanensis*
	**L naam*
	**L paidjan*
	L pomona
	**L pyrogenes*
	**L sarmin*
	**L schuffneri*
	L sejroe
	**L semarang*
	**L sentot*
	**L wolffii*
Iraq	*L grippotyphosa*
Israel	*L canicola*
	L grippotyphosa
	L pomona
Japan	*L australis A*
	**L autumnalis*
	L bataviae
	L canicola
	**L hebdomadis*
	**L icterohaemorrhagiae*
	L pyrogenes
Korea	*L icterohaemorrhagiae*
Lebanon	*L grippotyphosa*
	L icterohaemorrhagiae
†Malaya	*L australis A*
	L australis B
	L autumnalis
	L ballum
ASIA	
Malaya—*contd*	*L bangkinang*
	L bataviae
	L benjamin
	L canicola
	L celledoni
	L djasiman
	L grippotyphosa
	L hebdomadis
	L hyos
	L icterohaemorrhagiae
	L javanica
	L medanensis
	L naam
	L poi
	L pomona
	L pyrogenes
	L schüffneri
	L semarang
	L sentot
	L wolffii
Pacific Islands	
Hawaii	*L canicola*
	L icterohaemorrhagiae
Okinawa	*L autumnalis*
	L hebdomadis
Samoa	*L australis A*
	L icterohaemorrhagiae
Thailand	*L autumnalis*
	L bataviae
AFRICA	
Algeria	*L icterohaemorrhagiae*
†Belgian Congo	*L australis A*
	L bataviae
	L benjamin
	L canicola
	L grippotyphosa
	L hyos
	L icterohaemorrhagiae
	L pomona
	L wolffii

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA		AMERICAS	
Egypt	*L grippotyphosa*	West Indies—*continued*	
	L icterohaemorrhagiae		
	L sejroe	Trinidad	*L icterohaemorrhagiae*
Kenya	*L grippotyphosa*	South America	
French Morocco	*L icterohaemorrhagiae*	Argentina	*L canicola*
			L hyos
			L icterohaemorrhagiae
			L pomona
French West Africa	*L icterohaemorrhagiae*	Brazil	*L australis B*
			L canicola
Madagascar	*L grippotyphosa*		*L icterohaemorrhagiae*
	L pomona		
Tunisia	*L grippotyphosa*	Chile	*L canicola*
	L icterohaemorrhagiae		*L icterohaemorrhagiae*
			L pomona
		Ecuador	*L icterohaemorrhagiae*
AMERICAS		French Guiana	*L icterohaemorrhagiae*
North America			
Canada	*L ballum*		
	L icterohaemorrhagiae		
	L pomona		
Mexico	*L icterohaemorrhagiae*	AUSTRALASIA	
U.S.A	*L autumnalis*	†Australia	**L australis A*
	L ballum		**L australis B*
	L bataviae		*L canicola*
	L canicola		**L celledoni*
	L grippotyphosa		**L hyos*
	L icterohaemorrhagiae		*L icterohaemorrhagiae*
	L pomona		*L medanensis*
	L sejroe		**L mini*
			**L pomona*
West Indies			
Cuba	*L canicola*	New Zealand	*L canicola*
	L grippotyphosa		*L icterohaemorrhagiae*
	L icterohaemorrhagiae		*L pomona*
Guadeloupe	*L icterohaemorrhagiae*	Papua and New Guinea	*L andaman A*
†Puerto Rico	*L ballum*		*L bataviae*
	L bataviae		*L hyos*
	L canicola		Autumnalis serogroup
	L icterohaemorrhagiae		Hebdomadis serogroup
			Pyrogenes serogroup

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*
ASIA	
Indonesia	*L australis A*
	L autumnalis
	**L bangkinang*
	**L bataviae*
	**L benjamin*
	L canicola
	**L cynopteri*
	**L djasiman*
	L grippotyphosa
	**L hardjo*
	L hebdomadis
	L icterohaemorrhagiae
	**L javanica*
	**L mankarso*
	**L medanensis*
	**L naam*
	**L paidjan*
	L pomona
	**L pyrogenes*
	**L sarmin*
	**L schuffneri*
	L sejroe
	**L semarang*
	**L sentot*
	**L wolffii*
Iraq	*L grippotyphosa*
Israel	*L canicola*
	L grippotyphosa
	L pomona
Japan	*L australis A*
	**L autumnalis*
	L bataviae
	L canicola
	**L hebdomadis*
	**L icterohaemorrhagiae*
	L pyrogenes
Korea	*L icterohaemorrhagiae*
Lebanon	*L grippotyphosa*
	L icterohaemorrhagiae
†Malaya	*L australis A*
	L australis B
	L autumnalis
	L ballum

Continent and Country	*Serotype of Leptospire*
ASIA	
Malaya—*contd*	*L bangkinang*
	L bataviae
	L benjamin
	L canicola
	L celledoni
	L djasiman
	L grippotyphosa
	L hebdomadis
	L hyos
	L icterohaemorrhagiae
	L javanica
	L medanensis
	L naam
	L poi
	L pomona
	L pyrogenes
	L schuffneri
	L semarang
	L sentot
	L wolffii
Pacific Islands	
Hawaii	*L canicola*
	L icterohaemorrhagiae
Okinawa	*L autumnalis*
	L hebdomadis
Samoa	*L australis A*
	L icterohaemorrhagiae
Thailand	*L autumnalis*
	L bataviae
AFRICA	
Algeria	*L icterohaemorrhagiae*
†Belgian Congo	*L australis A*
	L bataviae
	L benjamin
	L canicola
	L grippotyphosa
	L hyos
	L icterohaemorrhagiae
	L pomona
	L wolffii

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA		AMERICAS	
Egypt	*L grippotyphosa* *L icterohaemorrhagiae* *L sejroe*	West Indies—*continued* Trinidad	*L icterohaemorrhagiae*
Kenya	*L grippotyphosa*	South America Argentina	*L canicola* *L hyos* *L icterohaemorrhagiae* *L pomona*
French Morocco	*L icterohaemorrhagiae*		
French West Africa	*L icterohaemorrhagiae*	Brazil	*L australis B* *L canicola* *L icterohaemorrhagiae*
Madagascar	*L grippotyphosa* *L pomona*		
Tunisia	*L grippotyphosa* *L icterohaemorrhagiae*	Chile	*L canicola* *L icterohaemorrhagiae* *L pomona*
		Ecuador	*L icterohaemorrhagiae*
AMERICAS North America		French Guiana	*L icterohaemorrhagiae*
Canada	*L ballum* *L icterohaemorrhagiae* *L pomona*		
Mexico	*L icterohaemorrhagiae*	AUSTRALASIA	
USA	*L autumnalis* *L ballum* *L bataviae* *L canicola* *L grippotyphosa* *L icterohaemorrhagiae* *L pomona* *L sejroe*	†Australia	**L australis A* **L australis B* *L canicola* **L celledoni* **L hyos* *L icterohaemorrhagiae* *L medanensis* **L mini* **L pomona*
West Indies			
Cuba	*L canicola* *L grippotyphosa* *L icterohaemorrhagiae*	New Zealand	*L canicola* *L icterohaemorrhagiae* *L pomona*
Guadeloupe	*L icterohaemorrhagiae*	Papua and New Guinea	*L andaman A* *L bataviae* *L hyos* Autumnalis serogroup Hebdomadis serogroup Pyrogenes serogroup
†Puerto Rico	*L ballum* *L bataviae* *L canicola* *L icterohaemorrhagiae*		

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	Serotype of Leptospire	Continent and Country	Serotype of Leptospire
ASIA		ASIA	
Indonesia	L australis A	Malaya—contd	L bangkinang
	L autumnalis		L bataviae
	*L bangkinang		L benjamin
	*L bataviae		L canicola
	*L benjamin		L celledoni
	L canicola		L djasiman
	*L cynopteri		L grippotyphosa
	*L djasiman		L hebdomadis
	L grippotyphosa		L hyos
	*L hardjo		L icterohaemorrhagiae
	L hebdomadis		L javanica
	L icterohaemorrhagiae		L medanensis
	*L javanica		L naam
	*L mankarso		L poi
	*L medanensis		L pomona
	*L naam		L pyrogenes
	*L paidjan		L schuffneri
	L pomona		L semarang
	*L pyrogenes		L sentot
	*L sarmin		L wolffii
	*L schuffneri		
	L sejroe	Pacific Islands	
	*L semarang	Hawaii	L canicola
	*L sentot		L icterohaemorrhagiae
	*L wolffii		
		Okinawa	L autumnalis
Iraq	L grippotyphosa		L hebdomadis
Israel	L canicola	Samoa	L australis A
	L grippotyphosa		L icterohaemorrhagiae
	L pomona		
		Thailand	L autumnalis
Japan	L australis A		L bataviae
	*L autumnalis		
	L bataviae		
	L canicola		
	*L hebdomadis		
	*L icterohaemorrhagiae	AFRICA	
	L pyrogenes	Algeria	L icterohaemorrhagiae
Korea	L icterohaemorrhagiae	†Belgian Congo	L australis A
			L bataviae
Lebanon	L grippotyphosa		L benjamin
	L icterohaemorrhagiae		L canicola
			L grippotyphosa
†Malaya	L australis A		L hyos
	L australis B		L icterohaemorrhagiae
	L autumnalis		L pomona
	L ballum		L wolffii

* Country in which this serotype was first isolated
† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA		AMERICAS	
Egypt	*L grippotyphosa* *L icterohaemorrhagiae* *L sejroe*	West Indies—*continued* Trinidad	*L icterohaemorrhagiae*
Kenya	*L grippotyphosa*	South America	
French Morocco	*L icterohaemorrhagiae*	Argentina	*L canicola* *L hyos* *L icterohaemorrhagiae* *L pomona*
French West Africa	*L icterohaemorrhagiae*	Brazil	*L australis B* *L canicola* *L icterohaemorrhagiae*
Madagascar	*L grippotyphosa* *L pomona*	Chile	*L canicola* *L icterohaemorrhagiae* *L pomona*
Tunisia	*L grippotyphosa* *L icterohaemorrhagiae*	Ecuador	*L icterohaemorrhagiae*
AMERICAS North America		French Guiana	*L icterohaemorrhagiae*
Canada	*L ballum* *L icterohaemorrhagiae* *L pomona*		
Mexico	*L icterohaemorrhagiae*	AUSTRALASIA	
U.S.A	*L autumnalis* *L ballum* *L bataviae* *L canicola* *L grippotyphosa* *L icterohaemorrhagiae* *L pomona* *L sejroe*	†Australia	**L australis A* **L australis B* *L canicola* **L celledoni* **L hyos* *L icterohaemorrhagiae* *L medanensis* **L mini* **L pomona*
West Indies Cuba	*L canicola* *L grippotyphosa* *L icterohaemorrhagiae*	New Zealand	*L canicola* *L icterohaemorrhagiae* *L pomona*
Guadeloupe	*L icterohaemorrhagiae*	Papua and New Guinea	*L andaman A* *L bataviae* *L hyos* Autumnalis serogroup Hebdomadis serogroup Pyrogenes serogroup
†Puerto Rico	*L ballum* *L bataviae* *L canicola* *L icterohaemorrhagiae*		

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	Serotype of Leptospire	Continent and Country	Serotype of Leptospire
ASIA		ASIA	
Indonesia	L. australis A	Malaya—contd	L. bangkinang
	L. autumnalis		L. bataviae
	*L. bangkinang		L. benjamin
	*L. bataviae		L. canicola
	*L. benjamin		L. celledoni
	L. canicola		L. djasiman
	*L. cynopteri		L. grippotyphosa
	*L. djasiman		L. hebdomadis
	L. grippotyphosa		L. hyos
	*L. hardjo		L. icterohaemorrhagiae
	L. hebdomadis		L. javanica
	L. icterohaemorrhagiae		L. medanensis
	*L. javanica		L. naam
	*L. mankarso		L. poi
	*L. medanensis		L. pomona
	*L. naam		L. pyrogenes
	*L. paidjan		L. schuffneri
	L. pomona		L. semarang
	*L. pyrogenes		L. sentot
	*L. sarmin		L. wolffii
	*L. schuffneri		
	L. sejroe	Pacific Islands	
	*L. semarang	Hawaii	L. canicola
	*L. sentot		L. icterohaemorrhagiae
	*L. wolffii		
		Okinawa	L. autumnalis
Iraq	L. grippotyphosa		L. hebdomadis
Israel	L. canicola	Samoa	L. australis A
	L. grippotyphosa		L. icterohaemorrhagiae
	L. pomona		
		Thailand	L. autumnalis
Japan	L. australis A		L. bataviae
	*L. autumnalis		
	L. bataviae		
	L. canicola		
	*L. hebdomadis		
	*L. icterohaemorrhagiae	AFRICA	
	L. pyrogenes	Algeria	L. icterohaemorrhagiae
Korea	L. icterohaemorrhagiae	†Belgian Congo	L. australis A
			L. bataviae
Lebanon	L. grippotyphosa		L. benjamin
	L. icterohaemorrhagiae		L. canicola
			L. grippotyphosa
†Malaya	L. australis A		L. hyos
	L. australis B		L. icterohaemorrhagiae
	L. autumnalis		L. pomona
	L. ballum		L. wolffii

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*
AFRICA	
Egypt	*L. grippotyphosa* *L. icterohaemorrhagiae* *L. sejroe*
Kenya	*L. grippotyphosa*
French Morocco	*L. icterohaemorrhagiae*
French West Africa	*L. icterohaemorrhagiae*
Madagascar	*L. grippotyphosa* *L. pomona*
Tunisia	*L. grippotyphosa* *L. icterohaemorrhagiae*
AMERICAS	
North America	
Canada	*L. ballum* *L. icterohaemorrhagiae* *L. pomona*
Mexico	*L. icterohaemorrhagiae*
U.S.A.	*L. autumnalis* *L. ballum* *L. bataviae* *L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae* *L. pomona* *L. sejroe*
West Indies	
Cuba	*L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae*
Guadeloupe	*L. icterohaemorrhagiae*
†Puerto Rico	*L. ballum* *L. bataviae* *L. canicola* *L. icterohaemorrhagiae*
AMERICAS	
West Indies—*continued*	
Trinidad	*L. icterohaemorrhagiae*
South America	
Argentina	*L. canicola* *L. hyos* *L. icterohaemorrhagiae* *L. pomona*
Brazil	*L. australis B* *L. canicola* *L. icterohaemorrhagiae*
Chile	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
Ecuador	*L. icterohaemorrhagiae*
French Guiana	*L. icterohaemorrhagiae*
AUSTRALASIA	
†Australia	**L. australis A* **L. australis B* *L. canicola* **L. celledoni* **L. hyos* *L. icterohaemorrhagiae* *L. medanensis* **L. mini* **L. pomona*
New Zealand	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
Papua and New Guinea	*L. andaman A* *L. bataviae* *L. hyos* Autumnalis serogroup Hebdomadis serogroup Pyrogenes serogroup

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
ASIA		ASIA	
Indonesia	*L australis A*	Malaya—*contd*	*L bangkinang*
	L autumnalis		*L bataviae*
	**L bangkinang*		*L benjamin*
	**L bataviae*		*L canicola*
	**L benjamin*		*L celledoni*
	L canicola		*L djasiman*
	**L cynopteri*		*L grippotyphosa*
	**L djasiman*		*L hebdomadis*
	L grippotyphosa		*L hyos*
	**L hardjo*		*L icterohaemorrhagiae*
	L hebdomadis		*L javanica*
	L icterohaemorrhagiae		*L medanensis*
	**L javanica*		*L naam*
	**L mankarso*		*L poi*
	**L medanensis*		*L pomona*
	**L naam*		*L pyrogenes*
	**L paidjan*		*L schuffneri*
	L pomona		*L semarang*
	**L pyrogenes*		*L sentot*
	**L sarmin*		*L wolffii*
	**L schuffneri*		
	L sejroe	Pacific Islands	
	**L semarang*	Hawaii	*L canicola*
	**L sentot*		*L icterohaemorrhagiae*
	**L wolffii*		
		Okinawa	*L autumnalis*
Iraq	*L grippotyphosa*		*L hebdomadis*
Israel	*L canicola*	Samoa	*L australis A*
	L grippotyphosa		*L icterohaemorrhagiae*
	L pomona		
		Thailand	*L autumnalis*
Japan	*L australis A*		*L bataviae*
	**L autumnalis*		
	L bataviae		
	L canicola		
	**L hebdomadis*		
	**L icterohaemorrhagiae*	AFRICA	
	L pyrogenes	Algeria	*L icterohaemorrhagiae*
Korea	*L icterohaemorrhagiae*	†Belgian Congo	*L australis A*
			L bataviae
Lebanon	*L grippotyphosa*		*L benjamin*
	L icterohaemorrhagiae		*L canicola*
			L grippotyphosa
†Malaya	*L australis A*		*L hyos*
	L australis B		*L icterohaemorrhagiae*
	L autumnalis		*L pomona*
	L ballum		*L wolffii*

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA		AMERICAS West Indies—*continued*	
Egypt	*L. grippotyphosa* *L. icterohaemorrhagiae* *L. sejroe*	Trinidad	*L. icterohaemorrhagiae*
Kenya	*L. grippotyphosa*	South America Argentina	*L. canicola* *L. hyos* *L. icterohaemorrhagiae* *L. pomona*
French Morocco	*L. icterohaemorrhagiae*	Brazil	*L. australis B* *L. canicola* *L. icterohaemorrhagiae*
French West Africa	*L. icterohaemorrhagiae*	Chile	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
Madagascar	*L. grippotyphosa* *L. pomona*	Ecuador	*L. icterohaemorrhagiae*
Tunisia	*L. grippotyphosa* *L. icterohaemorrhagiae*	French Guiana	*L. icterohaemorrhagiae*
AMERICAS North America Canada	*L. ballum* *L. icterohaemorrhagiae* *L. pomona*	AUSTRALASIA †Australia	**L. australis A* **L. australis B* *L. canicola* **L. celledoni* **L. hyos* *L. icterohaemorrhagiae* *L. medanensis* **L. mini* **L. pomona*
Mexico	*L. icterohaemorrhagiae*	New Zealand	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
U.S.A.	*L. autumnalis* *L. ballum* *L. bataviae* *L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae* *L. pomona* *L. sejroe*	Papua and New Guinea	*L. andaman A* *L. bataviae* *L. hyos* Autumnalis serogroup Hebdomadis serogroup Pyrogenes serogroup
West Indies Cuba	*L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae*		
Guadeloupe	*L. icterohaemorrhagiae*		
†Puerto Rico	*L. ballum* *L. bataviae* *L. canicola* *L. icterohaemorrhagiae*		

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*
ASIA	
Indonesia	*L australis A*
	L autumnalis
	**L bangkinang*
	**L bataviae*
	**L benjamin*
	L canicola
	**L cynopteri*
	**L djasiman*
	L grippotyphosa
	**L hardjo*
	L hebdomadis
	L icterohaemorrhagiae
	**L javanica*
	**L mankarso*
	**L medanensis*
	**L naam*
	**L paidjan*
	L pomona
	**L pyrogenes*
	**L sarmin*
	**L schuffneri*
	L sejroe
	**L semarang*
	**L sentot*
	**L wolffi*
Iraq	*L grippotyphosa*
Israel	*L canicola*
	L grippotyphosa
	L pomona
Japan	*L australis A*
	**L autumnalis*
	L bataviae
	L canicola
	**L hebdomadis*
	**L icterohaemorrhagiae*
	L pyrogenes
Korea	*L icterohaemorrhagiae*
Lebanon	*L grippotyphosa*
	L icterohaemorrhagiae
†Malaya	*L australis A*
	L australis B
	L autumnalis
	L ballum
ASIA	
Malaya—*contd*	*L bangkinang*
	L bataviae
	L benjamin
	L canicola
	L celledoni
	L djasiman
	L grippotyphosa
	L hebdomadis
	L hyos
	L icterohaemorrhagiae
	L javanica
	L medanensis
	L naam
	L poi
	L pomona
	L pyrogenes
	L schuffneri
	L semarang
	L sentot
	L wolffi
Pacific Islands	
Hawaii	*L canicola*
	L icterohaemorrhagiae
Okinawa	*L autumnalis*
	L hebdomadis
Samoa	*L australis A*
	L icterohaemorrhagiae
Thailand	*L autumnalis*
	L bataviae
AFRICA	
Algeria	*L icterohaemorrhagiae*
†Belgian Congo	*L australis A*
	L bataviae
	L benjamin
	L canicola
	L grippotyphosa
	L hyos
	L icterohaemorrhagiae
	L pomona
	L wolffi

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA Egypt	*L. grippotyphosa* *L. icterohaemorrhagiae* *L. sejroe*	AMERICAS West Indies—*continued* Trinidad	*L. icterohaemorrhagiae*
Kenya	*L. grippotyphosa*	South America Argentina	*L. canicola* *L. hyos* *L. icterohaemorrhagiae* *L. pomona*
French Morocco	*L. icterohaemorrhagiae*		
French West Africa	*L. icterohaemorrhagiae*	Brazil	*L. australis B* *L. canicola* *L. icterohaemorrhagiae*
Madagascar	*L. grippotyphosa* *L. pomona*	Chile	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
Tunisia	*L. grippotyphosa* *L. icterohaemorrhagiae*		
		Ecuador	*L. icterohaemorrhagiae*
AMERICAS North America Canada	*L. ballum* *L. icterohaemorrhagiae* *L. pomona*	French Guiana	*L. icterohaemorrhagiae*
Mexico	*L. icterohaemorrhagiae*	AUSTRALASIA †Australia	**L. australis A* **L. australis B* *L. canicola* **L. celledoni* **L. hyos* *L. icterohaemorrhagiae* *L. medanensis* **L. mini* **L. pomona*
U.S.A.	*L. autumnalis* *L. ballum* *L. bataviae* *L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae* *L. pomona* *L. sejroe*		
West Indies Cuba	*L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae*	New Zealand	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
Guadeloupe	*L. icterohaemorrhagiae*	Papua and New Guinea	*L. andaman A* *L. bataviae* *L. hyos* Autumnalis serogroup Hebdomadis serogroup Pyrogenes serogroup
†Puerto Rico	*L. ballum* *L. bataviae* *L. canicola* *L. icterohaemorrhagiae*		

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
ASIA		ASIA	
Indonesia	*L australis A*	Malaya—*contd*	*L bangkinang*
	L autumnalis		*L bataviae*
	**L bangkinang*		*L benjamin*
	**L bataviae*		*L canicola*
	**L benjamin*		*L celledoni*
	L canicola		*L djasiman*
	**L cynopteri*		*L grippotyphosa*
	**L djasiman*		*L hebdomadis*
	L grippotyphosa		*L hyos*
	**L hardjo*		*L icterohaemorrhagiae*
	L hebdomadis		*L javanica*
	L icterohaemorrhagiae		*L medanensis*
	**L javanica*		*L naam*
	**L mankarso*		*L poi*
	**L medanensis*		*L pomona*
	**L naam*		*L pyrogenes*
	**L paidjan*		*L schuffneri*
	L pomona		*L semarang*
	**L pyrogenes*		*L sentot*
	**L sarmin*		*L wolffii*
	**L schuffneri*		
	L sejroe	Pacific Islands	
	**L semarang*	Hawaii	*L canicola*
	**L sentot*		*L icterohaemorrhagiae*
	**L wolffii*		
		Okinawa	*L autumnalis*
Iraq	*L grippotyphosa*		*L hebdomadis*
Israel	*L canicola*	Samoa	*L australis A*
	L grippotyphosa		*L icterohaemorrhagiae*
	L pomona		
		Thailand	*L autumnalis*
Japan	*L australis A*		*L bataviae*
	**L autumnalis*		
	L bataviae		
	L canicola		
	**L hebdomadis*		
	**L icterohaemorrhagiae*	AFRICA	
	L pyrogenes	Algeria	*L icterohaemorrhagiae*
Korea	*L icterohaemorrhagiae*	†Belgian Congo	*L australis A*
			L bataviae
Lebanon	*L grippotyphosa*		*L benjamin*
	L icterohaemorrhagiae		*L canicola*
			L grippotyphosa
†Malaya	*L australis A*		*L hyos*
	L australis B		*L icterohaemorrhagiae*
	L autumnalis		*L pomona*
	L ballum		*L wolffii*

* Country in which this serotype was first isolated

† See Table III for additional strains not fully identified

TABLE VII—*continued*

Continent and Country	*Serotype of Leptospire*	*Continent and Country*	*Serotype of Leptospire*
AFRICA		AMERICAS	
Egypt	*L. grippotyphosa* *L. icterohaemorrhagiae* *L. sejroe*	West Indies—*continued* Trinidad	*L. icterohaemorrhagiae*
Kenya	*L. grippotyphosa*	South America	
French Morocco	*L. icterohaemorrhagiae*	Argentina	*L. canicola* *L. hyos* *L. icterohaemorrhagiae* *L. pomona*
French West Africa	*L. icterohaemorrhagiae*	Brazil	*L. australis B* *L. canicola* *L. icterohaemorrhagiae*
Madagascar	*L. grippotyphosa* *L. pomona*		
Tunisia	*L. grippotyphosa* *L. icterohaemorrhagiae*	Chile	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
		Ecuador	*L. icterohaemorrhagiae*
AMERICAS North America		French Guiana	*L. icterohaemorrhagiae*
Canada	*L. ballum* *L. icterohaemorrhagiae* *L. pomona*		
Mexico	*L. icterohaemorrhagiae*	AUSTRALASIA	
U.S.A.	*L. autumnalis* *L. ballum* *L. bataviae* *L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae* *L. pomona* *L. sejroe*	†Australia	**L. australis A* **L. australis B* *L. canicola* **L. celledoni* **L. hyos* *L. icterohaemorrhagiae* *L. medanensis* **L. mini* **L. pomona*
West Indies Cuba	*L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae*	New Zealand	*L. canicola* *L. icterohaemorrhagiae* *L. pomona*
Guadeloupe	*L. icterohaemorrhagiae*	Papua and New Guinea	*L. andaman A* *L. bataviae* *L. hyos* Autumnalis serogroup Hebdomadis serogroup Pyrogenes serogroup
†Puerto Rico	*L. ballum* *L. bataviae* *L. canicola* *L. icterohaemorrhagiae*		

* Country in which this serotype was first isolated

† See Table III for additional strains not fully ident

haemorrhagiae, the rat brought a new human disease into Britain

The assumption that leptospires originated in South East Asia and thence spread outwards in recent geological times could explain why only two serotypes are found in Britain, which was part of the continent of Europe until about 5000 B C Up to that time the rodents in Europe, including Britain, were presumably not infected with leptospires, and only the serotypes *carried by the rat and the dog have been introduced into* Britain since then

On the emergence of new serotypes, Broom quoted the suggestion of Gsell (1949) that adaptation to an unwonted carrier host may lead to the appearance of a new serotype if the proteins of the two hosts differ in chemical constitution Whether or not this occurs can be proved only by observation and experiment

The distribution of serotypes as at present known throughout the world is shown by countries in Table VII

LEPTOSPIROSIS IN MAN

CHAPTER V

EPIDEMIOLOGY PART I
ROUTE AND MEANS OF INFECTION

Cutaneous Infection Laboratory Infections Mucous Membranes Bathing and Involuntary Immersion Alimentary Tract Case to Case Transmission Trans Placental Transmission

Introduction

Although Chapters V and VI deal primarily with the epidemiology of Weil s disease, many of the factors involved are common to all forms of leptospirosis Where it has seemed appropriate, examples to illustrate certain general points have been taken from the epidemiology of other serotypes Special factors which influence the epidemiology of the other forms are considered in the sections in which the individual serotypes are treated

Weil (1886) believed that infection took place through the alimentary canal Inada *et al* (1916) were at first of the same opinion but later were satisfied that infection could take place through the shaven but macroscopically unbroken skin of guineapigs Although they were able to infect animals through the mucous membrane of the alimentary canal, massive doses of infected liver tissues were used for the purpose

Cutaneous Infection

In studying 55 cases among coal miners, Inada *et al* noted only a few instances directly suggesting cutaneous origin, but they stated that the cutaneous route was the probable means of entry because of the following facts (1) the incidence was greater in certain parts of the coal mine than in others, (2) there were many cases in wet and few in dry mines, (3) the infection took place more easily if the skin was injured, (4) coal miners were liable to abrasions of the skin and also to skin lesions caused by working with the feet in water



Fig 17

Veterinarians at work

In many countries veterinarians have been infected by different serotypes of leptospires Supplied by the Central Office of Information

anglers, and we have records of a number of cases of Weil's disease among workers in watercress beds

Opinions differ as to whether leptospires can pass through normal unabraded skin Inada *et al* (1916) concluded from their experiments with guineapigs that the organisms could do so, although a higher proportion of infections was obtained when the skin was damaged The question is to some extent academic rather than practical because microscopical abrasions might provide adequate portals of entry Some experimental evidence which may have a bearing on this question was brought forward by Varvello (1940) who worked with human volunteers Infection was produced in a man whose leg had been immersed for two hours in water to which a culture of *L bataviae* had been added No infection occurred in a man whose hand was so immersed for one hour

In some of the cases described by Welcker (1938) infection apparently took place through the intact skin It is however possible that the surface of the skin was damaged during unsuccessful efforts to kill the leptospires by the application of disinfectants immediately after the accident

ANIMAL BITES —The occurrence of Weil's disease in two men who had been bitten by rats 7 to 9 days previously was noted by Ido *et al* (1916) A number of similar cases has since been reported following bites of both albino and wild rats (Uhlenhuth and Zimmermann, 1933, Wahab, 1940) Schüffner and Bohlander (1942 a & b) were led to the discovery of *Microtus arvalis* as the carrier of *L grippotyphosa* because children bitten by these voles developed mud fever A case of Weil's disease following a dogbite was reported by Jacobsen (1936), and one of canicola fever by Lereboullet, Kolochine-Erber and Castel (1949)

Mechanical transmission to man by one animal from another animal has apparently occurred occasionally Wigmore and Denning (1936) reported an infect on b [illegible]

[illegible]

on the lip by a rat Alston and Brown (1937) quoted another example of a ferret bite in which the same mechanical transmission of infection was probable, since they could not demon-

General experience confirms the belief that, for all serotypes, the skin is the most common portal of entry, especially through cuts, abrasions, bites or sodden surfaces Experimental evidence in favour of this view was obtained by van Thiel and Engelbrecht (1957) using strains of *L icterohaemorrhagiae* and *L grippotyphosa* which had become nonvirulent through prolonged subculture They found that agglutinins appeared in the serum of human volunteers when the nonvirulent leptospires were applied to the scarified skin No antibodies developed when the skin was intact, or when the leptospires were introduced into the nasal and buccal cavities and the stomach Infection of guineapigs by the nasal route could be effected with virulent but not with nonvirulent strains and therefore the authors suggested that virulent strains may have greater powers of penetration

In London sewer workers, Alston and Brown (1937) found a significant difference in the incidence of Weil's disease between the two classes of workmen employed The builder's labourers who break up and handle old brick work or concrete covered with slime suffer more abrasions than the flushers who clean the walls of the sewers (Fig 15) It was found that during eighteen months in 1934-35, the builder's men showed a case incidence ten times higher than the flushers Several of the workers suffering from Weil's disease had gashed or abraded wounds when they were admitted to hospital, and these wounds had been made at a time within the incubation period of the disease

Skin wounds are common also among fish cleaners (Fig 16), butchers and workers in slaughter houses and meat packing establishments—whose occupations all carry a special risk of leptospirosis The infection of veterinarians is no doubt caused in the same way (Fig 17)

Condition of the Skin —A wet or sodden state of the skin, even if it is not obviously injured, is considered by Borg Petersen (1944 a) to aid the penetration of leptospires Such conditions may explain the liability to contract infection through the feet by treading on infected ground during work in rice fields, sugar plantations, pig sties, during military duties, or by walking on muddy banks after bathing in fresh-water pools Esseveld (1937) drew attention to the risk run by

days the ticks were ground up and injected into guineapigs, which died with typical signs of leptospirosis These authors do not place any aetiological significance on their findings, but Schlossberger and Langbein (1952) consider that *O moubata* may transmit leptospirosis to dogs and other domestic animals In their first experiments they fed nymphs of this tick on a guineapig infected with *L icterohaemorrhagiae* Half an hour after the feed a batch of ticks was ground up and the fluid obtained injected into a guineapig Other batches of ticks were dealt with in the same way at intervals up to 39 days after feeding In every case the animals became infected, showing that *L icterohaemorrhagiae* can survive and retain its virulence in ticks for at least 39 days In another experiment, eggs laid by ticks which had had two infective feeds were washed, ground and injected into a guineapig This animal also died of Weil's disease, from which it appeared that the leptospires not only survive in the gut but also penetrate into the ovaries

A method of infecting ticks by feeding them on the air sac of infected chick embryos was described by Burgdorfer and Pickens (1954), and was used in an extensive series of experiments by Burgdorfer (1956) He found that from 30 to 50 per cent of the third and fourth larval stages as well as adult *O turicata* became carriers of *L pomona* after an infective feed Leptospires could be seen in the gut contents of the ticks for the first two or three days, but not later In ticks which showed a persistent infection, leptospires passed through the gut wall and multiplied in the haemolymph They then invaded the coxal organs salivary glands, and excretory and genital systems Numerous leptospires were present in the coxal fluid from about the eighth day, and some ticks remained infective for as long as 232 days

Leptospires were seen in sections of the ovaries and egg follicles of several female ticks However, inocula prepared from several thousand freshly deposited eggs and from the progeny of infected females failed to infect guineapigs Thus Burgdorfer, unlike Schlossberger and Langbein, found no evidence that infection was transmitted to the next generation of ticks.

In spite of these occasional successful transmission experi-

strate agglutinins in the ferret's blood In these three cases the intervals between the bite and the onset of illness were 5, 7 and 10 days respectively It is natural to assume that the teeth of the dog and the ferret were contaminated by contact with the infected viscera of the rats

INSECT TRANSMISSION—Noguchi (1918 b) attempted to transmit *L icterohaemorrhagiae* by means of insects, and he included in his experiments the larvae and adult stages of *Culex pipiens*, larvae of *Musca domestica* and *Caliphora vomitoria* and adult wood ticks (*Dermacentor andersoni*) The insects were allowed either to bite infected guineapigs or to feed on infective material None of the insects became carriers and he concluded that they played no part as intermediate hosts

Kingsbury (1928) likewise failed to transmit infection by bites of *Aedes argenteus* and *A albipictus*, as did Gay and Sellards (1927) by *A aegypti* The latter workers found however that leptospires survived for about three weeks in some of the mosquitoes Although no infection resulted when the insects bit clean guineapigs, successful transmissions were sometimes obtained when the ground-up bodies of the same mosquitoes were injected

More recently, Kunert and Schmidtke (1952) attempted to infect *Musca domestica, Calliphora erythrocephala* and *Lucilia sericata* with *L icterohaemorrhagiae, L grippotyphosa* and *L canicola* They found that no leptospires were present in the adult flies which developed from infected larvae or pupae However, if adult flies were fed on infective material, leptospires might survive in the crop and on the outer cuticle for at least 26 hours Flies might thus transport leptospires passively, and serve as a means of contaminating food or water The authors suggested that these findings might explain the occurrence of certain sporadic cases of leptospirosis of obscure origin

Bed bugs (*Cimex lectularius*) were fed on infected guineapigs by Blanchard, Lefrou and Laigret (1923) The bugs transmitted the infection to clean guineapigs at their next feed 38 days later Similar results with bed bugs were obtained by Bonne (1924 a) but he could not obtain infections at intervals greater than 48 hours after the infective feed

Varela, Curbelo, Vasquez and Guzmán Neira (1954) allowed *Ornithodorus nicollei* to feed on infected guineapigs After 3

Although the wound was immediately washed with spirit, and a short prophylactic course of penicillin was given (see p. 224), leptospirosis developed 10 days after the accident. As in Schuffner's case (noted above) the diagnosis was confirmed by demonstrating leptospires microscopically in a specimen of blood taken on the day of onset. The strain also grew in cultures from the same specimen.

Mucous Membranes

As was noted above, human laboratory infections have followed the accidental contamination of the conjunctival sac and the mouth with infective material. Stavitsky (1945) had similar results in an experimental study with guineapigs, rats and mice, but he failed to transmit infection through the intact nasal mucosa though van Thiel (1934) had found it a satisfactory route. On the other hand, Varvello (1940) obtained no infections either when diluted culture was instilled into the eye or when it was used repeatedly to rinse out the mouth. Donatien and Gayot (1951) tried to infect guineapigs *via* the mouth and nose, but failed except on one occasion by the latter route. Varvello's success after passing culture into the stomach by tube may therefore have been due to abrasions of the nasal mucosa.

Bathing and Involuntary Immersion

An association between Weil's disease and bathing (Fig. 18) was recognised by Jaeger (1892) and has been amply confirmed by later observers (*e.g.*, Schüffner, 1934; Walch-Sorgdrager, 1939; Broom, 1951 a; Fuhner, 1950 b; Gauld, Crouch, Kaminsky, Hullinghorst, Gochenour and Yager, 1952). Schüffner thought that the higher incidence among male swimmers might be due to their greater addiction to the "crawl" stroke in which the whole face is repeatedly immersed under the water. As an alternative Campbell, Macrae, Manderson, Sumner and Broom (1950) suggested that boys might be less fastidious than girls in their choice of bathing sites, and so be more likely to bathe in infected pools.

The first proved case of Weil's disease in England occurred in a man who fell into the River Thames (Manson-Bahr, Wenyon and Brown, 1922). This mode of infection has been

most intensively studied by workers in the Netherlands where the widespread system of canals, many with rat-infested banks, provides an omnipresent potential source of the disease (Fig 19) Unpremeditated immersion, and also unsuccessful attempts at suicide by drowning, carry the special danger of violent struggling, which leads to the entry of water into the respiratory and alimentary tracts, as well as into the eyes

ASSOCIATION BETWEEN BATHING AND MENINGITIS LEPTOSPIROSA —It has long been noted that the so called 'pure meningeal form' of Weil's disease, without jaundice, occurs in a relatively high proportion of patients infected while bathing In these cases the mucous membranes of the eyes and naso pharynx are the probable portals of entry, and Buzzard and Wylie (1947) suggested that infection by this route might 'predispose to the meningeal form of the disease' Of the 5 cases of meningitis described by these observers only 3 had contracted the disease by bathing, but all were young, their ages ranging from 9 to 23 years We therefore examined our own records of 616 cases of true Weil's disease which occurred in the British Isles from 1947–53 inclusive to determine whether the age of the patient or the mode of infection was more closely correlated with the occurrence of meningitis leptospirosa The series included 113 cases due to bathing or accidental immersion in water, and 41 of these (35 per cent) were of the pure meningeal form Of the 503 cases infected in other ways, 80 (15 per cent) were of this form The age distribution of cases in the two series was however very different, as is shown in Table VIII The percentages of cases showing meningitis at different ages were therefore calculated, and are shown in Table IX The results of this analysis strongly suggest that, within any one age group, the proportion of cases of the pure meningeal form of Weil's disease is approximately the same, whether the mode of infection is by bathing or otherwise

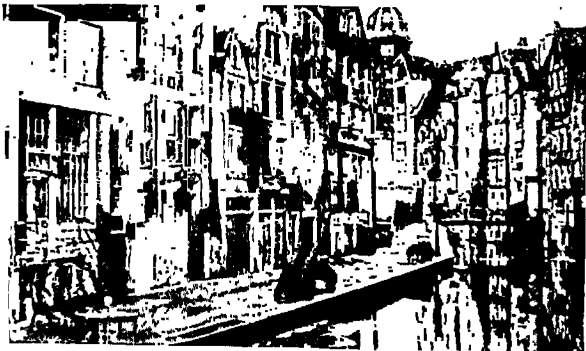
That factors other than age also play an important part was well illustrated by two of our cases The patients were brothers, aged 15 and 16 years, who bathed in the same river, and later developed Weil's disease about the same time One boy's illness manifested itself as meningitis leptospirosa, in the other the early symptoms suggested acute appendicitis, and



Fig 18

Bathing in fresh water

A cause of infection by different serotypes of leptospires in many countries
Supplied by the Central Office of Information



A canal in Amsterdam

Canals have been the source of many infections of Weil's disease in Amsterdam

the boy was saved from laparotomy only by the timely occurrence of a severe epistaxis

ALIMENTARY TRACT

A number of instances have occurred in which Weil s disease has apparently been contracted by drinking water or consuming food which had been contaminated Since the gastric secretion

TABLE VIII

AGE DISTRIBUTION OF CASES OF WEIL S DISEASE CONTRACTED BY BATHING AND OTHERWISE *

Age in Years	0-14	15-29	30 and over	Total
Bathing Cases	35 (31%)	50 (44%)	9 ( )	113
Other Cases	23 (5%)	94 (19%)	386 ( )	3
All Cases	58 (9%)	144 (24%)	414 ( )	616

* The figures in parentheses show the relative proportion [illegible] each age group

TABLE IX

PROPORTION OF CASES OF MENINGEAL FORM IN DIFFERENT AGE GROUPS

Age in Years	0-14	15-29	30 and over
Bathing Cases	60%	3 %	1
Other Cases	65%	30	1
All Cases	6 %	31%	11

is acid it seems unlikely that leptospires could survive to penetrate the mucous lining of the stomach but they may find portals of entry in the upper regions of the alimentary tract

DRINKING WATER —The water of a drinking fountain the source of which might have been contaminated by rats was considered by Jorge (1932) to be the cause of an epidemic of 1?8 cases of Weil s disease in Lisbon Another outbreak of 21 cases in an island in the Aegean Sea was described by

no pathological changes were present in the tissues and no leptospires could be demonstrated Nevertheless, the author believed that the foetus was infected

The case recorded by Lindsay and Luke (1949) also showed curious features An infant, normal at birth, developed jaundice 30 hours later and soon became listless, cyanotic and dyspnoeic Death ensued 14 hours after the first appearance of jaundice *Histological examination revealed extensive necrosis* of liver cells, and marked degeneration of the tubular epithelium in the kidneys In sections stained by silver impregnation methods, a few leptospires were seen in the liver and kidneys The mother showed no signs of illness either during her pregnancy, or postpartum A blood specimen taken two weeks after the confinement had agglutinin titres of 1/10,000 against both *L icterohaemorrhagiae* and *L canicola* Another sample obtained three months later reacted negatively with both serotypes The authors think that the mother had latent leptospirosis during the latter part of her pregnancy, but we agree with Gsell (1952) and do not consider the conclusion justified by the evidence

CHAPTER VI

EPIDEMIOLOGY PART II
INCIDENCE WITH REGARD TO SEX, AGE, OCCUPATION, SEASON AND YEAR

SEX

Such evidence as is available suggests that there is no difference between the sexes in susceptibility to leptospirosis When men and women work together under similar conditions of risk, the incidence is approximately the same Thus among fish workers in Aberdeen, Scotland, there were some 100 cases of Weil's disease in females and 80 in males from 1934–48 (Smith, 1949) During 1948, 1,476 females and 1,076 males were employed in the industry If one assumes that these figures can be taken as an estimate of the proportion of the two sexes at risk, the annual case incidence would be of the order of 4 5 per 1,000 for females and 4 8 for males

Babudieri and Bianchi's series (1940) of *L pomona* infections in Italian rice fields contained a preponderance of women Similarly, the epidemic among pea harvesters described by Popp (1950) comprised 172 females and 84 males Popp does not give the relative numbers at risk, but mentions that the majority of the workers were women

In Denmark, as was noted by Borg-Petersen (1949), infection with *L sejroe* shows an interesting temporal variation in sex incidence In August, during harvesting, which is carried out almost entirely by men, large numbers of cases occurred in males In the autumn the carrier hosts migrated from the fields to the farm buildings, and then the female incidence rose steeply

Canicola fever, which is frequently contracted in the home from dogs, provides another example of equal risk to the sexes Rosenberg (1951), who collected reports of 200 cases, noted that 44 per cent of the patients were females

In these instances men and women were equally at risk,

but most occupations and pastimes which carry a special risk of infection are mainly followed by men (Table X) For that reason, male cases largely predominate in most series of observations For example, the original patients described by Weil and by Landouzy (p 3–4) were men, and Hunter (1908) estimated that 90 per cent of cases were in males.

Table X

OCCUPATION OF 983 WEIL'S DISEASE CASES IN THE BRITISH ISLES FROM JULY 1933 TO JULY 1948 (INCLUSIVE)

Occupation	*Number*	*Per Cent of Total*
Fish Workers	216	22 1
Other Food Handlers		
Butcher, Slaughterer, Fishmonger, Tripe scraper	21	2 1
Coal Miners	139	14 2
Sewer Workers	79	8 1
Farm Workers	45	4 6
Gardeners	4	0 4
Builders	9	0 9
Army including P O W (6)	67	6 9
Navy	19	1 9
Air Force	11	1 1
Bathing or Paddling including Involuntary Immersion (7)	48	4 9
Workers in Water		
Canal, Gravel pit, Watercress beds, *River drainage*	16	1 6
Bite or Scratch by rat, dog, ferret	11	1 1
Laboratory Workers	2	0 2
Miscellaneous *e g*		
Ragwork, Bottle washing	[illegible]	3 0
Unrecorded or not yet analysed	[illegible]	26 9
		100 0

In an early … of … n and
(1917) … 1 7 …
(1939) in the … 11 .
of Weil's …
in men Our ow…
1951 a)

(95 per cent) Similarly in North Queensland Australia, the proportion of cases recorded by Derrick, Gordon, Ross, Doherty, Sinnamon, MacDonald and Kennedy (1954) was 6 women (4 per cent) and 146 men (96 per cent)

We have cited only a few examples to illustrate this point, but they serve to show that the high proportion of cases in males occurs in all parts of the world

Age

No age is immune from leptospirosis but it has been suggested that young children may be relatively less susceptible to infection, at least by some serotypes For instance, canicola fever is often contracted in the home from infective dog urine excreted on to the floor One would think that very young children playing on the floor would be as liable to become contaminated as the adults who tend the sick dogs and clean up the dejecta Yet Rosenberg (1951) who reviewed a series of 200 human infections with *L canicola* found few patients under 10 years of age

This is not in conformity with our findings as will be seen from Table XI In our series, 17 (14 per cent) of the patients suffering from canicola fever were below 10 years of age, as compared with 4 per cent of Weil's disease cases The actual ages of the children and the number of cases are shown in Table XII The two children aged 5 years and one aged 4 years were girls, the rest were boys

TABLE XII

AGE DISTRIBUTION OF 17 CASES OF CANICOLA FEVER IN CHILDREN UNDER TEN YEARS

Age in years	2	3	4	5	6	7	8	9	*Total*
No of cases	2	0	2	2	4	2	3	2	17

Table XI shows the age incidence of 881 cases of Weil's disease (ranging in age from 1½–84 years) and 118 of canicola fever, all of which occurred in the British Isles from 1940-55 It will be seen that the proportions of cases of Weil's disease rise continuously up to the fifth decade, whereas the highest proportion for canicola fever is reached in the fourth decade.

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Farm Workers	45	4 6
Gardeners	4	0 4
Builders	9	0 9
Army including P O W (6)	67	6 9
Navy	19	1 9
Air Force	11	1 1
Bathing or Paddling including Involuntary Immersion (7)	48	4 9
Workers in Water		
Canal		
Gravel pit	16	1 6
Watercress beds		
River drainage		
Bite or Scratch by rat dog ferret	11	1 1
Laboratory Workers	2	0 2
Miscellaneous e g		
Ragwork	29	3 0
Bottle washing		
Unrecorded or not yet analysed	267	26 9
	983	100 0

In an early Japanese series of fatal cases Kaneko and Okuda (1917) *recorded 36 males and 7 females* Walch-Sorgdrager (1939) in the Netherlands described only 40 cases (11 per cent) of Weil's disease in women, as compared with 323 (89 per cent) in men Our own two series of cases (Broom and Alston, 1948, Broom, 1951 a) comprised 34 women (5 per cent) and 620 men



Fig 20

Sugarcane cutting in Queensland Australia

Rat infestation of sugarcane has caused much leptospiral infection Reproduced from The Australian Sugar Industry—Some Facts and Figures by kind permission of the Agent General for Queensland



Fig 21

Transplanting rice seedlings in Italy

Working in wet rice fields causes leptospiral infections in many rice growing countries By Prof M Austoni Reproduced from Le Leptospirosi by kind permission of Tipografia del Seminario Padua

TABLE XI

AGE INCIDENCE OF LEPTOSPIROSIS BRITISH ISLES 1940–55

Age in Years	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	*Totals*
L. icterohaemorrhagiae	34 (3·8%)	133 (15·1%)	133 (15·1%)	168 (19·1%)	182 (20·6%)	130 (14·7%)	85 (9·6%)	14 (1·6%)	2 (0·2%)	881
L. canicola	17 (14%)	23 (19%)	21 (18%)	29 (25%)	17 (14%)	7 (6%)	5 (4%)	0	0	118

TABLE XIII

AGE INCIDENCE OF LEPTOSPIROSIS SWITZERLAND 1944–51

(after Gsell 1953)

Age in Years	1–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	Total
L. icterohaemorrhagiae	2	10	21	10	13	4	1	—	61
L. canicola	1	6	7	7	5	4	2	—	32
L. grippotyphosa	3	24	28	25	10	3	1	—	94
L. sejroe	—	15	15	13	7	4	—	—	54
L. australis A	3	11	9	3	3	5	—	—	34
L. pomona	9	84	113	53	32	6	3	2	301
L. hyos	—	20	30	12	9	5	3	—	79
L. autumnalis	—	—	1	—	1	—	—	—	2
TOTAL PERCENTAGE	17 (2·6)	170 (25·8)	224 (34·1)	123 (18·7)	80 (12·2)	31 (4·8)	10 (1·5)	2 (0·3)	657 (100)

Numbers in heavy type indicate age group with highest incidence



Fig 9

Sugarcane cutting in Queensland Australia

Rat infestation of sugarcane has caused much leptospiral infection Reproduced from The Australian Sugar Industry—Some Facts and Figures by kind permission of the Agent General for Queensland



Fig 10

Transplanting rice seedlings in Italy

Working in wet rice fields causes leptospiral infections in many rice growing countries By Prof M Austoni Reproduced from Le Leptospirosi by kind permission of Tipografia del Seminario Padua

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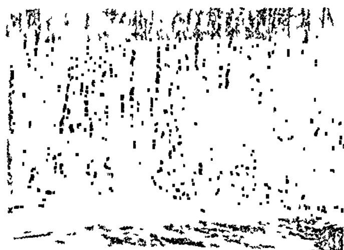
TABLE XIII

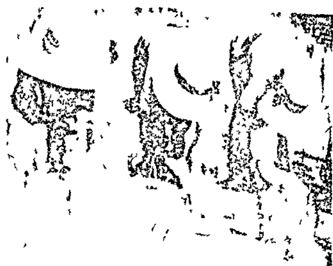
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L grippotyphosa	3	24	28	25	10	3	1	—	94
L sejroe	—	15	15	13	7	4	—	—	54
L australis A	3	11	9	3	3	5	—	—	34
L pomona	8	84	113	53	32	6	3	2	301
L hyos	—	20	30	12	0	5	3	—	79
L autumnalis	—	—	1	—	1	—	—	—	2
TOTAL PERCENTAGE	17 (2 6)	170 (25 8)	224 (34 1)	123 (18 7)	80 (12 2)	31 (4 8)	10 (1 5)	2 (0·3)	657 (100)

Numbers in heavy type indicate age group with highest incidence





Gsell's (1953) analysis of infections by the various [illegible] present in Switzerland is reproduced in Table XIII It [illegible] be noticed that the maximum incidence among the [illegible] patients is in the third and fourth decades or earlier.

Occupation

Leptospiral diseases are more definitely associated [illegible] certain occupations and pastimes than are most infective [illegible] (Alston, 1957) Except for canicola fever which is [illegible] by dogs, infection rarely occurs in the home unless the [illegible] are heavily infested with rodents Analyses show [illegible] highest proportion of cases occur among agricultural [illegible] and that they are most likely to be infected when [illegible] crops of grain, vegetables and sugar cane (Fig 20), [illegible] in occupations such as transplanting rice seedlings [illegible] working in wet conditions (Fig 21) Close [illegible] domestic animals also carries a special hazard [illegible] equally to persons who breed and care for them, to [illegible] and to those who handle and process carcases [illegible]

Workers in sewers and in wet coal mines [illegible] liable to infection, but many other occupations [illegible] building may on occasion be carried out under [illegible] where there is a high risk of infection Bathing [illegible] accidental immersion in fresh water, and military [illegible] wet swampy ground may also be hazardous in [illegible] Table XIV are set out the more important [illegible] pastimes associated with leptospirosis, together [illegible] countries and serotypes concerned

Season

Seasonal variations in incidence are a notable [illegible] infections with almost all serotypes It was noted [illegible] Ido *et al* (1917) that Weil's disease was common [illegible] of moderate temperature, and least often [illegible] hottest and coldest times of the year Taylor [illegible] stated that the incidence of leptospirosis in [illegible] Islands was highest during the period of [illegible] monsoon An association with wet weather [illegible] been noted and the relationship was care[illegible]

C

Table XIV

OCCUPATIONAL RISK OF LEPTOSPIRAL INFECTION ACCORDING TO SEROTYPES

(This Table includes mainly the better known serotypes i.e. those in Table II)

General Category	*Detailed Form*	*Serotypes*
Agricultural especially field work	General farm work including grain harvesting	*L. andaman A* *L. australis B* *L. autumnalis* *L. bataviae* *L. grippotyphosa* *L. hebdomadis* *L. icterohaemorrhagiae* *L. pomona* *L. pyrogenes* *L. sejroe*
	Rice cultivation	*L. andaman A* *L. australis B* *L. ballum* *L. bataviae* *L. canicola* *L. grippotyphosa* *L. icterohaemorrhagiae* *L. poi* *L. pomona* *L. saxkoebing*
	Sugarcane cutting	*L. australis A* *L. australis B* *L. celledoni* *L. icterohaemorrhagiae* *L. naam* Esposito type Kremastos type Robinson type Valbuzzi type
	Vegetable growing	*L. grippotyphosa*
Animal contact	Veterinary work	*L. canicola* *L. hyos* *L. icterohaemorrhagiae*
	Pig breeding	*L. canicola* *L. hyos* *L. pomona* *L. sejroe*
	Cattle raising	*L. grippotyphosa* *L. hyos* *L. pomona*
	Dog Kennel work	*L. icterohaemorrhagiae*

for the varying relationship between seasons and months in different parts of the world

Broom (1951 a) prepared histograms (Fig 22) showing the onset of illness by months for 472 cases of Weil's disease which occurred in England and Wales from 1947–50 Infections due

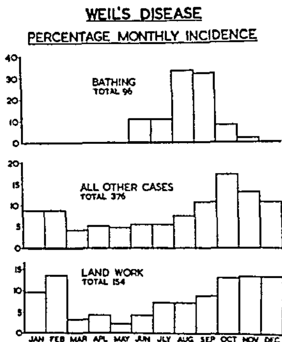


Fig 22

Percentage of monthly incidence of Weil's disease in England and Wales 1947 1950 By Dr J C Broom Reproduced from the British Medical Journal by kind permission

to bathing were naturally limited almost entirely to the summer months The seasonal variation was less marked for the other cases, but there was a distinct tendency for the numbers to increase in the late summer and autumn, and to fall during spring and early summer Amongst agricultural workers the incidence was high from October to February This may be due to an increase in the rat population around farm buildings

TABLE XIV—*continued*

General Category	*Detailed Form*	*Serotypes*
Fresh Water—*continued*	Watercress growers	*L icterohaemorrhagiae*
	Anglers	*L icterohaemorrhagiae* *L pomona*
Armed Forces	Army	*L australis A* *L australis B* *L autumnalis* *L bangkinang* *L bataviae* *L benjamin* *L canicola* *L celledoni* *L djasiman* *L grippotyphosa* *L hebdomadis* *L icterohaemorrhagiae* *L javanica* *L medanensis* *L poi* *L pomona* *L pyrogenes* *L schuffneri* *L wolffii*
	Navy	*L icterohaemorrhagiae*
	Air Force	*L icterohaemorrhagiae*
Laboratory *		*L alexi* *L autumnalis* *L ballum* *L grippotyphosa* *L hebdomadis* *L icterohaemorrhagiae* Javanica serogroup
Nursing *		*L grippotyphosa*

* Recorded cases

Queensland, Australia, by Derrick *et al* (1954) By estimating correlation coefficients these workers found that the best correlation was obtained when the incidence of cases was compared with the rainfall twelve days earlier

Borg Petersen (1949) found in Denmark that infections with *L icterohaemorrhagiae*, *L canicola* and *L sejroe* were more frequent in the second half of the year Similar observations have been recorded for other serotypes, when allowance is made

om the urine of rats The rats lived in the banks of neighbouring canals and visited the fields at night and in the early orning

In some occupations, in which conditions appear to change ttle throughout the year, seasonal variations in the incidence f Weil s disease occur and are difficult to explain Alston 1948) analysed 50 cases of Weil s disease which occurred in ondon sewer workers from 1934–45 Only 6 infections were ontracted in the months December to March as compared ith 44 during the other months, yet all forms of work in the ewers continue regularly all the year round Davidson and mith (1939) also noted variations in the incidence of Weil s isease among fish workers in Aberdeen Scotland They tated that infections occurred least often in February and larch and most frequently in August and September, although he fish trade operated at about the same activity throughout he year

It is not known whether carrier rats may possibly excrete eptospires more profusely during the winter Another possible xplanation is that rats obtain water more readily in the open luring the wetter months, and may not need to seek it elsewhere This might apply especially to sewers It is believed hat rats live and get their food in buildings, but enter the ewers by way of broken drain pipes to obtain water

Klarenbeek (1938) stated that one half of 57 dogs, which ontracted *I icterohaemorrhagiae* infections from 1935–37, leveloped the disease in September, October and November

Seasonal variations of canicola fever in dogs have also been *eported from some areas Thus Brede (1951) found in* Germany that the highest incidence of canine infections was n the late summer and winter He thought this finding might account for a similar seasonal incidence of human cases Broom and Joshua (1949) had recorded the same type of fluctuation in ngland from a study of 230 infected dogs, but from a much arger series Broom (1951 a) came to the conclusion that the ncidence remained comparatively constant throughout the year

YEAR

Variations in incidence from year to year have often been noticed sometimes for fairly obvious reasons For example,

when the harvest has been brought in from the fields Rats readily infest stacks of unthreshed grain, many are killed during threshing operations and the risk of contamination with infective urine is then high

Obvious reasons for these variations are often to be found in changes of occupation and outdoor sport at different seasons Good examples are bathing and fishing in fresh water, harvesting of different crops in countries where infected mice and voles are present, preparing the flooded rice fields and transplanting the seedlings, cutting sugarcane, etc

Wolff and Ruys (1953) compared the incidence of Weil's disease in Amsterdam during the three 6 year periods 1934–39 1940–45, 1946–51 In the first period there was a seasonal [illegible] August and September of cases due to bathing and [illegible] accidental falls into the [illegible] distributed throughout the year In the second period, the greatest incidence was in the winter months because of a great increase of accidental immersions due to the prohibition of lighting during the Second World War It is interesting to note that in this period there was an absence of Weil's disease in Amsterdam during the summer of 1944 owing to the brackishness of the canals to which sea water had been admitted In the third period, the preponderance of summer infections returned The number of cases due to water accidents was less than before the war, because fewer people fell into canals, but the risk of contracting Weil's disease during accidental immersion was the same as in the pre war period

Local conditions sometimes alter the period of highest incidence even when the same agricultural crop is cultivated For instance Mino (1942 a) traced the infection of workers by *L bataviae* in the rice fields in Italy to a great increase of mice (*Micromys minutus soricinus*) Epidemics tended to occur when the rice plants were strong enough to support the mice above the water This occurred at the end of June with consequent heavy contamination of the water and outbreaks of leptospirosis took place in July By contrast, Altava, Villalonga, Barrera and Marin (1953) found that in Spain the incidence was highest at harvest time when the fields were dry In this case the stalks of the rice plants were contaminated with *L icterohaemorrhagiae*

who studied variations in the incidence of the disease in England over a period of seventeen years His records showed that, quite often, a series of cases would occur in a particular locality within a comparatively short space of time and then no others would appear perhaps for years Meanwhile the cycle would be repeated in other areas

Borg Petersen (1949) associated an exceptionally large number of infections by *L sejroe* in Denmark during 1943 with a great increase of mice in that year At the time of the epidemic of leptospir

Saxony,

of *Micro*

of the mice in the affected areas were found to be carriers Olejnik and Shneyerson (1950) also found heavy infections of *Microtus guentheri* in an area in Israel where about 1,000 persons, chiefly growers of vegetables, were infected with *L grippotyphosa*

Altava, Barrera and Marin (1954) investigated an epidemic of Weil's disease among rice-field workers in Spain in 1952, and found that 50 per cent of *R norvegicus* and 100 per cent of *R frugivorus* were carriers of *L icterohaemorrhagiae* The following year the carrier rates were 19 per cent and 46 per cent respectively, and no epidemic of Weil's disease occurred

Kathe (1928) noted a high incidence of *L grippotyphosa infections in years of heavy rainfall* Also, Joshua (1949) attributed the low incidence of canine infections with *L canicola* in the autumn and winter of 1948–49 to unusually dry conditions in London, England

Fluctuations of incidence which occur in other circumstances cannot however be so readily explained For example, Sharp (1953) analysed the occurrence of cases of Weil's disease in three coal mines in Scotland from 1930–43 In one mine there were 13 cases in the years 1936–37, but no further infections occurred until 1942 in which year there were 8 cases The second mine had 14 cases over the whole period, but 8 occurred in 1942 In the third mine there were 8 cases from 1939–41 No further cases have been reported from any of these mines since 1943, although Mine No 1 continued in operation up to 1951, and Mine No 3 was still working in 1953 This was not an isolated occurrence because Sharp gave other instances of changes in incidence in coal mines in various parts of Scotland, and he showed also how the "accent on incidence" shifted among the different coal mining areas in Scotland during the period

Similar changes in the incidence of Weil's disease, not associated with coal mining, were noticed by Broom (1953 c)

leucocytes was seen in sections According to Veratti (quoted by Austoni, 1953) the lymphatic vessels are dilated, and contain histiocytes, erythrocytes and haemoglobin

INTERNAL ORGANS

At necropsy there is usually generalized jaundice, associated with lesions affecting the liver, kidneys, spleen and the circulatory system Damage to capillaries manifests itself as haemorrhages which may be present in all organs and tissues but are most marked under the skin, peritoneum and pleura and in the lungs, brain kidneys, and gastro intestinal tract

LIVER —The histological changes which occur in the early stages of Weil s disease were described by Ostertag (1950) from material obtained *during life* Unfortunately he did not record the day of disease on which the specimens were taken He character-[illegible]

increase in the number and size of Kupffer cells which contained ingested pigment and erythrocytes *After death* the liver may be normal in size or slightly enlarged and is firm on section The most constant and striking change is dissociation of the cord structure with separation of the hepatic cells which lie irregularly (Fig 23) There may be areas of necrosis around the central vein, but these are more common when death occurs during the early stages of illness The sinuses are congested and contain increased numbers of lymphocytes and polymorphs Small foci of infiltration of polymorphs and round cells are seen lying in a matrix of young fibrous tissue, sometimes near the smaller bile ducts The portal areas also show small degrees of cellular infiltration The cells around the hepatic veins usually contain large amounts of bile pigment The hepatic cells may show varying degrees of cloudy swelling but only minor amounts of fatty change, and evidences of regeneration are an outstanding feature Many of the cells contain two or more nuclei, and mitotic figures are not uncommon (Fig 24)

The severity of the changes in the liver vary considerably from one fatal case to another This can be explained by the

CHAPTER VII

MORBID ANATOMY AND HISTOLOGY, CLINICAL PATHOLOGY

MORBID ANATOMY AND HISTOLOGY

The pathological changes caused by leptospires have been most thoroughly studied in fatal cases of Weil s disease They were well described by Beitzke (1916) Pick (1917) Dawson *et al* (1917) McNee (1920) Buchanan (1927) Ashe Pratt Thomas and Kumpe (1941) and many later observers have confirmed their findings

Skin

Leptospires produce no inflammation at the site of entry either in the skin or mucous membranes In animal experiments on guineapigs and white rats Stavitsky (1945) showed that *L icterohaemorrhagiae* left the area of intradermal inoculation within half an hour and he found no evidence of phagocytosis of the organisms Volland and Brede (1951) found hyaluronidase in the culture fluid of nine serotypes and they speculated whether this enzyme might be a factor in the rapid dissemination of leptospires into the blood stream

Lymphatic Glands

Enlargement of lymphatic glands occurs in a variable proportion of cases the inguinal epitrochlear and cervical being most frequently affected At necropsy the mesenteric glands may be enlarged congested and even haemorrhagic (Taylor and Goyle 1931) Proliferation of the endothelial cells and phagocytosis of erythrocytes was noted by Buchanan (1927) in some of the fatal cases he examined An enlarged epitrochlear gland was excised on the eleventh day of illness by Troisier and Boquien (1930) and phagocytosis of polymorphonuclear

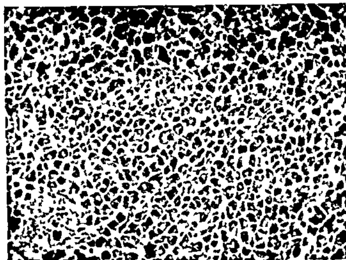


Fig 23

Liver from Weil's disease showing dissociation of liver cords and portal infiltration ( ) By Dr J C Broom and Dr K M Robertson Reproduced from Modern Practice in Infectious Fevers by kind permission of Butterworth & Co (Publishers) Ltd

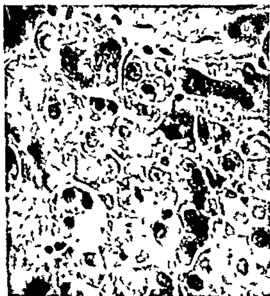


Fig 24

Higher magnification of part of Fig 23 showing binucleate cells and mitotic figures the latter indicated by arrows (600) By Dr J C Broom and Dr K M Robertson Reproduced from Modern Practice in Infectious Fevers by kind permission of Butterworth & Co (Publishers) Ltd

fact that death is more often caused by nephritis than by hepatitis Acute yellow atrophy is a very uncommon finding

The histological changes in the liver indicate hepatitis as the main cause of the jaundice in Weil s disease with intrahepatic obstruction to the flow of bile and haemolysis as minor factors This conclusion is supported by biochemical changes recorded on page 98

KIDNEY —The kidneys are often enlarged and may show the greenish brown staining found in cases of jaundice There may be small haemorrhages under the capsule in the tissue of the organ in the pelvis or in the perirenal fat

The histological changes present a very uniform picture in contrast to the variability of those found in the liver As a rule the glomeruli are little affected though Koppisch and Bond (1953) reported slight thickening of the basement membrane and cloudy swelling of cells of the capsular epithelium in 7 out of 11 cases examined by them The main damage however is confined to the cells of the tubules particularly the epithelium of the convoluted tubules (Fig 25) The cells show changes varying from cloudy swelling to complete necrosis with desquamation of cells into the lumen of the tubules Many of the cells contain bile pigment The tubules are often dilated particularly in the distal convoluted portions Cellular casts as well as blood haematin and hyaline casts many bile-stained are present in the medullary tubules (Fig 26) There is interstitial oedema which tends to become more diffuse as the length of time increases between onset and death Multiple haemorrhages are present and scattered areas of infiltration composed almost entirely of mononuclear cells

The lesion presents the features known as lower nephron nephrosis or diffuse distal tubular necrosis The changes are very similar to those seen in acute renal failure following abortion incompatible blood transfusion crush injuries severe trauma burns blackwater fever and some other conditions It is generally believed that these changes arise as a result of an over all reduction in blood flow caused by vaso constriction from within or sometimes from outside the kidney

Renal Shunt Mechanism —Trueta Barclay Daniel Franklin and Prichard (1947) postulated that the renal failure which occurred after crush injuries to the limbs might be due to



Fig 27

Kidney from Weil's disease showing ischaemia of cortex and dilated vessels in medulla (× 45)



Fig 28

Spleen from Weil's disease showing phagocytosis of fragmented erythrocytes (× 800) By Dr J C Broom and Dr K M Robertson Reproduced from Modern Practice in



Fig 2[illegible]



spasm of the renal arteries leading to anoxia and consequent impairment of kidney function They found experimentally that a change in the distribution of blood in the renal vessels of rabbits could be produced causing ischaemia of the cortex and corresponding congestion of the medulla This condition occurred because the blood was diverted from the greater part of the cortex and was 'short circuited or shunted' through the glomeruli situated in the layer of the cortex closest to the medulla or directly into the medullary vessels

This change of distribution of blood could be produced experimentally (1) by crushing the muscles of a limb (2) by stimulating the proximal end of the divided sciatic nerve the distal end of the splanchnic nerve or the nervous plexus surrounding the renal artery (3) by severe rapid haemorrhage (4) by administration of adrenalin pituitrin or pitressin in high dosage or (5) by the injection of staphylococcal toxin

In discussing the relevance of these experiments to blackwater fever and leptospirosis Maegraith (1948 1953) pointed out that there is no clear evidence of a shunt mechanism in the human kidney However some vascular alteration of this kind is a possible explanation of the renal lesions in acute renal failure in Weil's disease and a section showing ischaemia of the cortex with engorgement of the medullary vessels is shown in Fig 27 On the other hand Bull Jockes and Lowe (1950) attributed the anuria which followed abortion due to intra uterine douching to blood loss severe pain haemolysis and formation of blood casts and other causes without invoking the shunt mechanism The question is therefore not yet answered

Recently Sevitt (1956) has shown that cases of fatal uraemia with histological appearances of lower nephron nephrosis occurred without oliguria in patients suffering from severe burns This should be remembered as a possibility in Weil's disease and treatment by Bull's regime should be applied if necessary (p 190)

SPLEEN — The spleen may be normal in size or enlarged and diffluent There may be focal or diffuse haemorrhages into the pulp and an inflammatory reaction but the most distinctive appearance is the large number of fragmented erythrocytes many undergoing phagocytosis (Fig 28) There may also be reticulo endothelial hyperplasia and reduction of '

Cardiovascular System

As noted above, haemorrhages are an outstanding feature in severe cases and they are found in all organs and tissues The haemorrhages, which vary from petechiae to massive bleedings, are believed to be due to capillary damage, and not to changes in the blood For instance, Ashe *et al* (1941) found normal results for bleeding, clotting and prothrombin times, and also a normal platelet count

Dragert (1934) reported mural endocarditis of the ventricle in two fatal cases of Weil's disease This must be rare, but Sodeman and Killough (1951) found electrocardiographic evidence of temporary heart involvement in 9 out of 80 patients, all of whom were jaundiced and severely ill, although they finally recovered As a rule, apart from small haemorrhages only slight myocarditis is found

Associated with the vascular system may be mentioned the various forms of rash which are found in many types of leptospirosis, especially the milder They may resemble the rashes of scarlet fever, measles typhoid fever, or be of other mixed forms

Voluntary Muscle

Lesions affecting single muscle fibres or small groups of adjacent fibres were noted by Beitzke (1916) and were fully described by Ashe *et al* (1941) The gastrocnemius muscle is most often affected, but others are sometimes involved The gross appearance of the muscle is not changed though punctate haemorrhages may sometimes be visible

In sections, the condition is first seen as a loss of cross-striation at certain points of individual muscle fibres. Then follow swelling, loss of longitudinal striation, vacuolation and hyalinization (Fig 29) Most of the damaged tissue is absorbed so that the fibre may show constrictions at some points The nuclei of the sarcolemma then proliferate, and there is an infiltration of histiocytes, plasma cells and polymorphs

Sheldon (1945) carried out a series of biopsies throughout the acute phase of illness and also during convalescence and found that regeneration of muscle took place from about the seventeenth day onwards There was no scarring or fibrosis

except in very severe cases In a later paper Sheldon (1953) reported that by means of the fluorescent antibody technique of Coons and Kaplan (1950) he could demonstrate the presence of leptospiral antigen in the areas of muscular degeneration The early occurrence of the lesion was demonstrated by Wylie (1946 b) in a patient suffering from Weil's disease (Fig 30) Vacuolation of muscle fibres and invasion of the muscle bundle were seen in sections of a specimen taken three days after the onset of symptoms The agglutination reaction did not become positive until two days after the biopsy

Jeghers Houghton and Foley (1935) differentiated this lesion from Zenker's degeneration such as occurs in typhoid fever The latter affects mainly the adductor muscles of the thigh and the abdominal muscles it commonly produces haematomata involves all the fibres in large areas and leads to swelling of the fibres without resorption and shrinkage

Other Lesions

Less common lesions found clinically or postmortem, include adrenal failure and the Waterhouse-Friderichsen syndrome leading to death (Jackson and Oleesky, 1946), haemoptysis and consolidation of a portion of a lung as reported for instance by Abellán Ayala (1948) and profuse haemorrhage into the gastro intestinal tract

Postmortem Appearances in Milder Forms

In the milder forms of leptospirosis the sites and forms of damage are similar to those of the more fatal forms and at postmortem similar changes are found For instance, in the first of the rare fatal cases of human canicola fever Wolff van Dam and Minkenhof (1951) reported that the blood urea on the fifth day (6 days before death) was 379 mg per 100 ml Anuria was present for a few days and the naked eye and microscopical appearances in the liver and the kidney were the same as in infection by *L icterohaemorrhagiae* The calf muscles showed necrosis inflammation and regeneration of muscle fibres. There were some congenital abnormalities but it was not considered that these increased the danger of the disease By contrast in another of the early reports of fatal canicola fever Weetch Colquhoun and Broom (1949) found

that chronic nephritis, following high blood pressure and albuminuria during pregnancy 16 years earlier, was largely responsible for the uraemia which developed The non protein nitrogen of the blood was 284 mg per 100 ml on the eighteenth day and the patient died on the twenty-fourth day Bukh (1940) reported a severe infection by *L canicola*, with threatened anuria from which the patient recovered after the blood urea had reached 280 mg per 100 ml

The morbid anatomy and histology of leptospiral infections has been studied in many species of animals, and in general the lesions found in fatal infections are similar to those in human beings Reference to them is made in Chapters XII XVI and XVII

CLINICAL PATHOLOGY

Haematology

Different degrees of normocytic normochromic anaemia occur with erythrocyte counts of 2 5 to 5 million per c mm and haemoglobin of 50 to 90 per cent In severe cases there is almost always a polymorphonuclear leucocytosis rising some times to 25,000 per c mm or higher The changes in the leucocyte count in infectious hepatitis are discussed in the section on differential diagnosis (p 173) Platelet counts, and the bleeding, clotting and prothrombin times are within normal limits A raised sedimentation rate is a constant finding, for instance, Hutchison, Pippard, Gleeson-White and Sheehan (1946) found readings of 20 to 40 mm in 1 hour (corrected Wintrobe) in all of 17 cases up to the eighth week of illness and figures of under 10 mm were not found until the tenth week, confirming the slow convalescence A high sedimentation rate in the early stage in predominantly meningeal forms of leptospiral infections helps to distinguish them from lymphocytic meningitis due to filterable viruses (Scheid, 1949)

The Wassermann Test

Some writers have reported positive Wassermann tests in serum or cerebrospinal fluid in Weil's disease, and Costa and Troisier (1916 c) stated that this is one of the acute infective conditions in which a 'false' Wassermann reaction may be

found Sladden (1939) in reporting nine cases of Weil's disease in South Wales noted that the Wassermann test was positive in one serum, weakly positive in two, and positive in two specimens of cerebrospinal fluid These positive reactions disappeared in some of these cases when sera were retested later

We have carried out Wassermann tests on the serum of 60 patients while they were ill with Weil's disease and found all except one to be negative in a routine form of test with 3 M H D of complement The exception was a serum which gave a weak reaction only The significance of this reaction was in doubt, but on the evidence of the remaining tests we do not consider that the Wassermann test is made positive in Weil's disease

TESTS OF RENAL FUNCTION

It was recognized early in the study of leptospirosis that death is most commonly due to renal failure In almost all forms of leptospirosis there is evidence of a toxic effect on the kidneys, shown by albuminuria In the more severe cases, bile, blood and cellular, granular or hyaline casts occur in the urine, the specific gravity of which increases and the amount decreases Baumann (1952) suggested that big initial doses of penicillin might liberate much toxin from dead leptospires and precipitate anuria or, alternatively, that anuria might be a sensitivity reaction to the drug

The concentration of urea in the blood is one of the best guides to prognosis in general and to the day-to-day course of events We have analysed (Table XV) blood urea concentrations (in mg per 100 ml) in 69 fatal jaundiced cases, 200 jaundiced cases which were not fatal and 90 nonicteric cases In the first group the average of the figures was 343 mg with a range from 50 to 800 mg and only four were below 150, in the second group the average was 148 mg with a range from 20 to 640 mg, and only four were 400 or above, in the third group the average was 63 mg with a range from 20 to 228 mg, and only seven were over 150 The results are also shown in Fig 31, in which the ordinate scale of the upper diagram has been reduced to one half of that used for the other two

In guineapigs—which react to leptospiral infection in a way

similar to human beings—Wylie (1946 a) showed that hepatic lesions are much less important than renal lesions in severe and fatal infections

Tests of Liver Function

Ashe *et al* (1941) pointed out that the stools in Weil's disease are rarely acholic, even in the most intensely jaundiced patients, and therefore the jaundice cannot be due solely to obstruction of the larger biliary tracts Similarly, in the vast majority of serious cases *the severity of the* jaundice is out of proportion to the extent of blood destruction in the spleen, or of haemorrhages in the tissues, and therefore haemolysis does not appear to be the chief factor Their conclusion—aided by histological findings—*was that hepatitis is the main* cause, and that obstruction to bile capillaries and haemolysis are secondary.

This general opinion has been supported by liver function tests in six cases by Chinn, Roth and Moore (1951) and in one patient, very fully investigated, by Sterling (1950) In Sterling's work the patient had a severe illness, but recovered Specimens were taken on 11 different days between the tenth and

Table XV

PERCENTAGE DISTRIBUTION OF HIGHEST OBSERVED CONCENTRATION OF BLOOD UREA IN 90 NONICTERIC CASES, 200 NON-FATAL ICTERIC CASES AND 69 FATAL ICTERIC CASES OF WEIL'S DISEASE

	Highest Concentration of Blood Urea in Steps of 50 mg /100 ml															
	Under 50	50–99	100–149	150–199	200–249	250–299	300–349	350–399	400–449	450–499	500–549	550–599	600–649	650–699	700–749	750 and over
90 Nonicteric cases	57.5	26.5	9	5.5	1	0	0	0	0	0	0	0	0	0	0	0
200 Non fatal icteric cases	21	26	15.5	15.5	12	6.5	4	2.5	0	0.5	1	0	0.5	0	0	0
69 Fatal icteric cases	1.5	1.5	3	6	14	8.5	*0	17.5	8.5	8	1.5	4.5	6	0	3	4.5

ninety-fifth days of illness Significant findings in the serum were a high proportion of direct-reacting bilirubin, cephalin flocculation 3+ on most days and alkaline phosphatase up to 10 Bodansky units The electrophoretic pattern of the serum proteins showed diminished albumin and an increase of

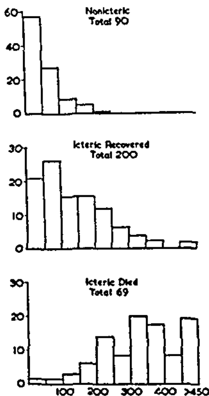


Fig 31

Histograms showing the distribution of blood urea levels in Weil's disease

gamma-globulin and of alpha-2-globulin, the first two changes in serum proteins are usual in hepatic dysfunction and the third may be caused by any febrile illness, as acute pneumonia Sterling's conclusions were that in Weil's disease the liver shows regurgitation jaundice due to liver cell necrosis—using the terminology of Rich (1930)

Cerebrospinal Fluid

Examination of the cerebrospinal fluid during life or afte death shows in most forms of leptospirosis that there is som degree of meningitis during the first week of the illness anc later, even in many patients who have no cranial symptom beyond headache For example, Swan and McKeon (1938 found a cell count of from 21 to 300 per c mm in 7 out of 8 patients with Weil's disease during the first or second week Meningitis in subclinical or clinical degree may be considered the most frequent manifestation of leptospiral infection, and in most of the less virulent types it is the most frequently recognized evidence of the disease An exception to this prevalence of meningitis has been found recently in Malaya (Trimble, 1957)

The fluid does not become purulent, the cells rarely exceed 2 000 per c mm and at first are mainly or partly polymorpho nuclear, but lymphocytes may greatly predominate later The protein may be increased up to 80 mg per 100 ml, and the concentration of glucose is within normal limits In contrast, Rubie and Mohun (1949) examined cerebrospinal fluid from a series of cases of tuberculous meningitis and found the glucose concentration to be reduced to between 5 and 45 mg per 100 ml In pyogenic meningitis it is more markedly decreased, and may even be absent The fluid may be bile-stained, and Cargill and Beeson (1947) pointed out that this occurs more often and at an earlier stage in leptospirosis than in other forms of jaundice, and so provides a useful diagnostic sign, this has been confirmed by several other observers At postmortem examination there may be meningeal congestion and small haemorrhages in the brain substance

CHAPTER VIII

CLINICAL ASPECTS OF SEVERE LEPTOSPIROSIS

As Exemplified by Leptospirosis Ictero-haemorrhagica (Weil's Disease)

INTRODUCTION

In this Chapter Weil's disease is taken as illustrating the full general pattern of leptospirosis In Chapter IX a detailed clinical account is given of canicola fever, as an example of the milder forms In Chapters X and XI which deal with the other serotypes, the clinical descriptions are briefer, but attention is paid to unusual features

In distinguishing the severity of leptospiral infections, the presence of jaundice and its degree are two of the most useful clinical characters By this criterion *L icterohaemorrhagiae* is the most virulent serotype and next are *L andaman A*, *L bataviae* and *L pyrogenes* Serotypes such as *L canicola*, [illegible]

haemorrhagiae mild or inapparent infections may occur Serological tests indicate that there is no subclinical infection among the general population, but it is found among persons frequently exposed to the risk of infection For example, Alston and Brown (1935) demonstrated agglutinins and protective antibodies in the blood of 9 out of 45 healthy London sewer workers who said that they had never had jaundice Similar findings were made by Smith and Davidson (1936) in 36 out of 193 fish workers in Aberdeen, Scotland, and by Mason (1938) in 6 out of 11 rat catchers in Liverpool, England

Mild unjaundiced cases were known to the Japanese discoverers of the causative organism, and Stokes *et al* (1917) reported that 60 per cent of patients in the British Army in

Cerebrospinal Fluid

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Mild unjaundiced cases were known to the Japanese discoverers of the causative organism, and Stokes *et al* (1917) reported that 60 per cent of patients in the British Army in

Flanders did not develop jaundice Schüffner (1934) found the same percentage in an epidemic in the Netherlands The unjaundiced cases are very rarely fatal, and they include a large number in which meningitis is the most prominent feature, and also others in which the signs and symptoms are even less well localized and approximate to those usually attributed to influenza We have diagnosed atypical infections by a rising agglutinin titre in persons such as sewer workers, and Stewart and Witts (1944) refer to four similar cases

The clinical picture is due to leptospiraemia, with selective involvement of the kidneys, liver, meninges and sometimes the lungs The leptospiraemia gives rise to malaise, pyrexia general and often severe debility, and muscular pains especially in the calves of the legs The renal involvement results in albuminuria, diminished secretion or suppression of urine vomiting and sometimes haematuria The hepatitis causes abdominal pain, anorexia, vomiting and jaundice with bile in the urine Severe headache is due to the meningitis and is aggravated by nephritis Involvement of the lungs leads to bronchitis or bronchopneumonia

TABLE XVI

SYMPTOMS AND SIGNS RECORDED IN 600 CASES OF WEIL'S DISEASE IN THE BRITISH ISLES

Feature	*Per cent*
Onset acute	62
Onset gradual	38
Jaundice	74
Haemorrhages	55
Headache	87
Muscle tenderness	69
Injected eyes	72
Meningitis clinically recorded	40
Neck stiffness	34
Albuminuria	75
Renal casts	40
Relapse second pyrexia (200 cases)	32

The physical signs vary according to the severity of the illness In mild cases the appearance may be that of a patient suffering from an illness resembling influenza, possibly with albuminuria and meningismus In severe cases the picture will be that of an extremely ill, jaundiced patient with conjunctival injection, neck stiffness, hepatomegaly, sometimes splenomegaly,

albuminuria, haematuria, reduction of urinary secretion and haemorrhages in the eyes and skin

Table XVI was compiled from the records of 600 cases of Weil's disease treated in various hospitals in Great Britain. It shows the proportion of cases in which certain signs and symptoms occurred, and our description of Weil's disease is based on these and on the reports of other observers.

INCUBATION PERIOD

The period of incubation of infections by *L. icterohaemorrhagiae* was accurately recorded for a number of cases by Schüffner (1934) He found that, in 37 cases due to falling into infected water, the incubation period varied from 4 to 19 days, and that in 31 (86 per cent) it was from 7 to 13 days In the series of laboratory infections reviewed by Welcker (1938) incubation periods were of 2 to 3 4, 6, 7, 8 (four times), 9, 10 and 11 days

Infections due to bites by animals also give reliable evidence of incubation time Thus Weil's disease resulting from rat bites occurred after 7 days (Bie, 1939), 7 to 9 days (Ido *et al*, 1916), 9 and 14 days (Alston and Brown, 1937) The bite of a dog which had just killed a rat was followed by disease in 7 days (Wigmore and Denning, 1936) and bites by ferrets preceded the disease by 5, 7 and 10 days (Alston and Brown, 1937)

Instances of very short incubation periods include the case of a doctor in Welcker's series who was taken ill 2 to 3 days after contaminating his eye with some infected liver which he was grinding, also the first case proved bacteriologically in the British Isles—a seaman who became ill 3 days after falling into the River Thames (Manson-Bahr, Wenyon and Brown, 1922). Two more instances of incubation periods lasting 2 days are given by Cattaneo (1929) and Fabini and Estrella (1938)

In the case of other serotypes for which accurate incubation periods have been obtained similar findings have been recorded. For instance, in a group of men injected with *L grippotyphosa* for fever treatment of nervous disease Korthof (1932) found incubation periods of 5 to 9 days Durand, Giroud, Larrive and Mestrallet (1936 a), who used swineherd's disease (before it was known to be due to *L. pomona*) for the same purpos

found that the incubation time varied from 6 to 12 days In the accidental laboratory infection with *L. ballum* reported by Wolff, Bohlander and Ruys (1949) the onset of symptoms occurred about 9 days after the patient had been scratched by an infected mouse Another laboratory infection, which occurred as the result of contamination of an eye with culture had an incubation period of 12 days (Alexander *et al*, 1952) The infecting strain was a serotype named by the authors *L. alexi*, but no details of its serological characteristics were given

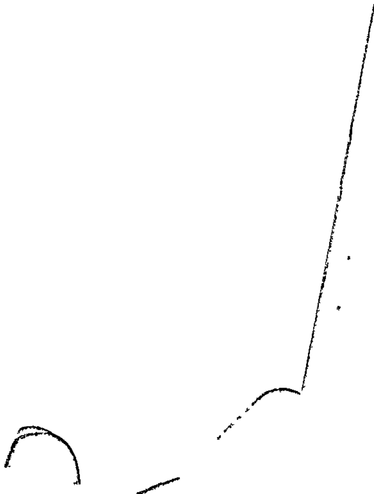
ONSET

In most types of leptospirosis the onset of illness is frequently sudden and sharp, and is often accompanied by rigors or shivering One patient known to us said that his illness began so suddenly that he could refer it to a particular moment while he was riding his bicycle Davidson and Smith (1936) reported a sudden onset in all of 40 cases of Weil's disease which they studied, but it would certainly be unwise to consider this feature a diagnostic sign We have seen several patients whose illness began more gradually, allowing them to remain at work for the first few days, but they became quickly and fatally worse about the fourth or fifth day The disease may even begin in a treacherously mild way In 600 cases of Weil's disease which Broom analysed, 62 per cent began suddenly (Table XVI)

SYMPTOMS AND COURSE OF ILLNESS

The course of the illness may be divided into three stages corresponding to leptospiraemia, development of antibodies and convalescence with leptospiruria

First Stage—The first stage occupies approximately the first week, and in varying order the following symptoms may occur during the first few days—malaise, general and often severe debility, fever rising as high as 104°F (40°C.), headache, and muscular pains in the calves or elsewhere in the body Alimentary symptoms are often prominent and may include nausea, vomiting with haematemesis, or abdominal pain sufficiently severe to suggest a surgical emergency Respiratory



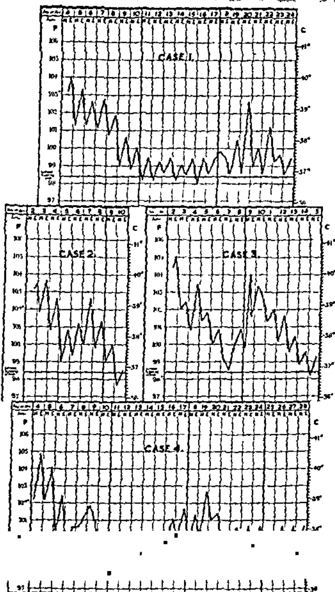


Fig 32

Temperature charts of Adolph Weil's original four patients Redrawn from the 'Deutches Archiv fur Klinische Medizin' by kind permission

symptoms are less frequent, but cough and thoracic pain sometimes occur with blood-stained sputum denoting bronchitis and bronchopneumonia, sore throat is frequent

Jaundice usually appears in the latter part of the first week and may be the earliest sign to distinguish Weil's disease from more common infectious fevers Conjunctival injection is frequent, but is not so important diagnostically as is sometimes stated, because it often closely resembles the conjunctivitis found in some acute upper respiratory infections or in catarrhal jaundice Haemorrhages in the skin and mucous membranes may begin at this time, if they are numerous the prognosis is serious, but the converse is not necessarily true because there may be few in fatal cases Debility may be very great

TABLE XVII

DAY OF ILLNESS ON WHICH DEATH OCCURRED FROM WEIL'S DISEASE IN 78 PATIENTS

Day of illness	6	7	8	9	10	11	12	13	14	15	16
No of deaths	2	1	9	8	8	10	8	9	6	3	1
			61 = 78% (days 8–15)								

Day of illness	17	18	19	20	21	22	23	24	25	26	27
No of deaths	2	1	1	1	1	1	1	1	1	2	1

Physical examination shows enlargement of the liver by palpation in a minority of cases, and of the spleen even less commonly, and the results of urine analysis and increase of blood urea indicate acute nephritis After an early leucopenia a polymorph leucocytosis is present Some of the physical signs of meningitis and inflammatory changes in the cerebrospinal fluid are almost always present to some degree

Death occurs most commonly in the second week but is earlier in a few very severe cases As was emphasized in the section on morbid anatomy, the rise of the blood urea—especially after it has reached 350 mg per 100 ml—is the best guide to prognosis

Table XVII shows the day of illness on which 78 patients died none of these died before the sixth or after the twenty-

seventh day, 61 of the patients (78 per cent of the total) died on the eighth to the fifteenth days *inclusive*

SECOND STAGE—The second stage also occupies about a week, and is the critical time in most of the serious infections The jaundice becomes deeper, the temperature often falls at first, but may rise to 102°F (39°C) again in the second part of the week In his original paper Weil showed temperature charts with and without this secondary rise (Fig 32) Subcutaneous and internal haemorrhages may be more numerous A rash occurs in about 10 per cent of patients and it may resemble that of measles, scarlet fever or typhoid fever, or it may be petechial or purpuric or macular with haemorrhage Herpes, especially near the mouth and nose, occurs in many patients and may be haemorrhagic Iritis, iridocyclitis, optic neuritis or papilloedema may be found, and retinal haemorrhages and paresis of the external muscles of the eye

Less common lesions found clinically or postmortem include suprarenal failure and the Waterhouse-Friderichsen syndrome, leading to death with large haemorrhages in both glands (Jackson and Oleesky, 1946) Haemoptysis and pulmonary consolidation were reported by Abellán Ayala (1948), and profuse haemorrhages into the gastro-intestinal tract, enceph *alitis, multiple neuritis or spondylitis sometimes occur* The most serious risk to life is increasing renal failure, with decrease or cessation of urinary excretion, rise of blood urea and accompanying heart failure and fall of blood pressure These may lead to coma and death The appearance of antibodies in the second half of this week is discussed in Chapter XII

THIRD STAGE—The third stage may be considered as occupying the third and any subsequent weeks of illness, merging into convalescence It is then that leptospires are most likely to be discovered in the urine The production of antibodies continues to increase as shown by titration in blood, urine and cerebrospinal fluid In general, the signs of illness abate, renal function recovers—sometimes associated with marked diuresis—and jaundice begins to lessen In approximately half the patients a rise of temperature of a few degrees occurs for a few days either in this period, *or during the latter* part of the second week *Usually this secondary increase of* fever is not accompanied by a relapse of symptoms, although

fresh areas of muscular degeneration have been found, and in some of the milder leptospiral infections meningitis is first manifested during this secondary rise of temperature Convalescence with more or less rapid fading of jaundice, and improvement of cardiac action and of general strength may be expected to be complete by the end of the sixth to the twelfth week of illness

OCULAR LESIONS

Ophthalmological features have been mentioned in the first and second stages of the illness but they rarely cause permanent injury to sight The lesions were fully described by Coutela (1948) and Gsell (1952) In one form or another—especially as conjunctivitis, episcleritis or iritis—inflammation of the eye is found during the first week in the majority of patients with the icteric or the anicteric form of the disease (Table XVI) Vascular congestion and some photophobia, but very little serous or purulent secretion and very little pain are the characters of this inflammation In addition, affection of the inner eye on one or both sides may occur as a complication of the illness or during a relapse or series of relapses from the second or third week up to a year later These late lesions have been studied most in infections of human beings and animals by serotypes other than *L icterohaemorrhagiae* but they have been found in the late stages of Weil's disease also At least nine serotypes have been found associated with them

The proportion of cases of Weil's disease which develops inflammation of the inner eye during convalescence or later is uncertain for lack of sufficiently frequent examination, and because the symptoms are often not marked, but in different series it has been found in from 5 to 100 per cent The lesions recorded have been uveitis involving the iris, ciliary body or choroid, opacities on the back of the cornea, turbidity of the aqueous humour, hypopyon, adhesions between lens and iris, opacities of the vitreous membrane, or rarely, optic neuritis It is only the more extreme degrees of inflammation that may lead to complete or permanent loss of vision

In a few instances involving various serotypes, leptospires have been cultured from the aqueous humour (Alexander *et al*, 1952, Strobel, 1952) and it is possible that some cases of late

uveitis may be allergic. Uveitis without a recognizable previous leptospiral infection has rarely if ever yet been proved in human beings to be due to leptospires. For example, we obtained negative agglutination reactions when samples of serum from 78 patients, suffering from various inflammatory conditions involving the uveal tract, were tested against *L. icterohaemorrhagiae*, *L. canicola*, *L. sejroe*, *L. pomona*, *L. grippotyphosa* and *L. bataviae*. (The relationship of periodic ophthalmia in horses to leptospirosis is discussed on p. 250.)

ILLUSTRATIVE CASE HISTORIES

A Severe Fatal Infection (Alston, 1935)

A man aged 52 was admitted to hospital on 18th November 1934. He had been employed since June 1934 in relaying sewers for which he worked in very old open trenches, and the sewers contained much slime and silt. He had done tunnelling work before, but not for some years. During October he cut a finger of the left hand on the edge of a broken drain pipe during employment, and this wound was dressed and he continued working.

On 11th November the patient felt very hot, and on the next day complained of severe headache and pains all over. There was a slight cough and a few bronchial râles, but no other physical signs of disease. The temperature was 102°F (38.9°C.). Sodium salicylate was prescribed. On the 13th and 15th the patient had been sleeping badly and the medicine was being vomited, even when diluted. On the 15th a little milk was retained and Medinal grains 5 was prescribed. The temperature on those days was 99.6° and 99°F. (37.6° and 37.2°C.). On the 17th the urine was dark red with blood and a little blood was coughed up, and the patient complained of itching, and jaundice was probably present. The temperature was 99.2°F. (37.3°C.). On the 18th the patient was jaundiced all over, there was much blood in the urine, and epistaxis and haematemesis occurred. The liver was enlarged and the stools were clay-coloured for the first time.

On Admission.—Delirium and slight twitchings were present and the temperature was 98°F. (36.7°C.). On the 19th the condition was almost the same, the conjunctivae were much

injected, epistaxis had recurred, and blood and bile were detectable in the urine Blood was vomited, and both altered and fresh blood were present in the stools The result of a blood count was red cells 2,670,000 per c mm , Hb 50 per cent , C I , 0 9 , leucocytes, 25,000 per c mm (polymorphs 91 per cent) On the 21st the condition became worse, restlessness and twitchings were more marked, and an estimation of blood urea showed 480 mg per 100 ml On the 22nd the patient died

At the time of admission the patient was questioned about the possibility of contact with rats in the course of his work He appeared to be sufficiently rational to appreciate the question and denied the likelihood of any such contact In view however of the severe and unusual symptoms which developed two or three days before death the possibility of leptospiral jaundice was entertained A specimen of blood serum was examined and the leptospiral adhesion test was found strongly positive

Postmortem Findings —(1) *Autopsy* Dark canary-yellow jaundice of skin and (less severe) of all serous surfaces , well-nourished, well-developed muscular man A few small recent abrasions on skin of chest probably due to scratching , laceration of nail of middle finger of left hand extending throughout its length Pale flabby myocardium Moderate general and coronary atheroma Slight bronchopneumonia, oedema and [illegible] capsule and in perirenal fat Slight haemorrhage in fatty tissue round both adrenals A few scattered haemorrhages in mucosa of stomach and throughout intestines Pale greenish-brown urine in bladder A few minute haemorrhages in mucosa of bladder Slight diffuse subdural haemorrhage and a few petechiae in cortex of oedematous brain

(2) *Histology* The following organs were examined histologically :—liver, kidney, spleen, adrenal, lung, brain, pituitary and parathyroid The sections were stained by Ehrlich's haematoxylin and eosin, by van Gieson's method, and liver, kidney and adrenal sections were stained by Levaditi's method for spirochaetes The macroscopic findings were confirmed

In addition there was an excess of polymorphs in the spleen, and slight fibrosis of the anterior lobe of the pituitary The changes in the liver and kidney warrant a more detailed description

In the liver the chief changes consisted of jaundice and degeneration of cells in the central parts of the lobules and in those of the mid-zonal regions The jaundice was less severe than that expected from the naked eye appearance, and the degeneration consisted of fatty change and necrosis of scattered individual cells There were patches of subacute inflammatory infiltration of the interstitial tissue of the kidney, the reaction consisting of plasma cells, lymphocytes and a few polymorphs The epithelium of the convoluted tubules was everywhere degenerate, many of the cells contained bile pigment, and in many tubules there were bile casts

Comment on Autopsy—Definite leptospires were not found but particularly in the kidney and adrenal there were structures resembling fragmented leptospires, and many granules that might well have been the remains of leptospires

A MODERATELY SEVERE NON FATAL INFECTION (Alston, 1935)

A man aged 35 was admitted to hospital on 11th July 1934 He had been employed for 3 years as a flusher in sewers The history of the case was that on 6th July, 5 days before admission, the illness had begun suddenly—like 'influenza'— with pains which started in the lower limbs and gradually extended upwards to the abdomen and between the shoulder blades The onset was so sudden that it could be referred to a particular moment while the patient was riding his bicycle On the following day he had some lower abdominal pain and began to vomit The vomiting was severe, and the vomit was dark and may have contained blood, there was no other haemorrhage Jaundice was noticed the day before admission to hospital The patient's health had previously been good So far as he knew he had not recently suffered any abrasion or cut of the skin, or a rat bite He stated that some of his fellow workers had been jaundiced, but they had not been ill enough to go to hospital

On Admission—The patient was a well-developed man with intensely jaundiced skin and conjunctivae He was moderately

ill, with a temperature of 100°F (37 8°C) and a pulse rate of 116 The tongue was dry and coated, the gall bladder tender, the liver not enlarged, and the spleen not palpable No purpura or petechiae were present No pathological state was detectable in the heart or lungs The urine contained bile pigment in large quantity, a trace of albumin and no sugar or acetone The stools were dark

For the first few days the patient suffered from continual vomiting and dehydration, but these conditions were relieved by enemata followed by rectal injections of glucose saline solution The general condition then improved, and temperature and pulse rate had returned to normal on the fifth day The jaundice and presence of bile in the urine took about a fortnight to subside On the fifth day in hospital a transient erythematous rash was seen, mainly on the limbs The patient left hospital on 2nd August The result of a blood count on 12th July was red cells 5,000,000 per c mm , Hb 100 per cent , C I 1 0, leucocytes 17,000 per c mm (polymorphs 87 per cent , lymphocytes 9 per cent , monocytes 4 per cent) The blood urea was 125 mg per 100 ml on 12th July, 145 mg on the 14th, and had returned to normal limits by the 19th The van den Bergh reaction gave an 'immediate direct' positive result up to 19th July, and a 'delayed direct' positive result on 27th July and 8th August The number of units by the indirect reactions was highest—54—on 14th July and was 4 on 8th August

Bacteriological Investigations—On 12th and 23rd July the urine was examined for leptospires by dark ground illumination but none was seen A guineapig was injected intraperitoneally on 12th July with the deposit from centrifuged urine, but no evidence of infection was detected Specimens of blood serum were obtained on 4th August and 12th September and both gave a strongly positive reaction in the adhesion test with *L icterohaemorrhagiae*

A Mild Infection

The patient, a sewer worker aged 33 became ill on 5th October 1947, with pain in the back and limbs, and attacks of shivering Two days later, in hospital, he showed slight jaundice and slight conjunctivitis The infection was a mild

one and the van den Bergh test showed 1 25 mg bilirubin per 100 ml of blood Penicillin (50,000 units every 3 hours) and antileptospiral serum (Burroughs Wellcome & Co, 40 ml per day) were given on the 8th, 9th and 10th October On the 10th, symptoms subsided and treatment was stopped Pyrexia *headaches and knee pains returned next day* *These ended* after 5 days treatment with penicillin, and returned again in a second relapse The symptoms disappeared, but a third relapse followed which in turn finally subsided after penicillin therapy During these relapses, in which the severe headaches were prominent, the cerebrospinal fluid showed 28 cells per c mm. on the twenty-seventh day of the illness, and 50 on the sixty-seventh day The serum did not agglutinate *L ictero haemorrhagiae* at the third day of the illness, but did so to a titre of 1/1,000 on the ninth day and 1/3,000 on the twentieth day The cerebrospinal fluid agglutinated *L icterohaemorrhagiae* to a titre of 1/30 on the twenty sixth day

Penicillin and serum are not now considered effective in *treatment of established infections (Chapter XIII)*

MENINGITIS LEPTOSPIROSA

Patients in whom meningitis is the most prominent feature of the infection, with or without a minor degree of jaundice make up a very large group As has been already stated meningitis is demonstrable clinically and by changes in the cerebrospinal fluid in practically all cases of the severe forms of infection, but the purely meningeal form is mild and very rarely fatal

French workers were the first to emphasize this type of the disease Costa and Troisier (1916 b) found it among French soldiers, and transmitted the infection to guineapigs from human cerebrospinal fluid They and others made a series of studies, and Troisier and Boquien (1933) who summarized the findings in the literature recorded 20 cases of leptospiral meningitis They emphasized the features of a sharp onset, *muscle pains, epigastric pains and headache* oliguria was frequent, with albuminuria in 40 per cent Some patients showed slight jaundice, *so providing a link with the usual form* of the disease Single or multiple relapses occurred, and all

the patients including children and adolescents recovered Later observers have confirmed these findings

Various indications of neurological involvement occur, including Kernig's sign, changes in reflexes, severe headache and weakness of muscles Pains in muscles may inhibit voluntary movement and lead to the mistaken suspicion of paresis Marked haematuria has occurred on the eleventh day of illness (Lereboullet, Kolochine-Erber and Lambert, 1951 b) Conjunctivitis, iritis and scleritis occur, and when the eyes are carefully examined uveitis is often found (Strobel, 1952) Skin rashes are seen as in the more jaundiced patients Since the infection is mild the leucocyte count may not be above 10,000 per c mm and the differential count may be normal Biochemical findings often confirm moderate changes in hepatic and renal function The changes in the cerebrospinal fluid are given on p 100

The temperature does not usually rise so high as in the more severe form, but it may show a second rise during the second or third week We have seen patients in whom moderately intense meningitis developed as a relapse during convalescence from a comparatively severe icteric form of the disease A unique case with severe jaundice and long lasting meningitis was described by Murgatroyd (p 118)

On rare occasions encephalitis is revealed (usually in conjunction with meningitis) by diplopia, nystagmus paralysis of cranial nerves, paraplegia, myoclonia, disturbance of sleep or psychical changes (Mach, 1944, Girard and Devic, 1949)

A Case of 'Pure' Meningitis

In 1947 we investigated an example of 'pure' meningitis due to *L icterohaemorrhagiae* in one of two brothers, who both had anicteric Weil's disease The boy who showed meningitis was aged 16 years He had frequently bathed during the summer with his brother in the river Stour, near Canterbury, England On 2nd August he complained of headache, and was admitted to hospital six days later There were no definite neurological signs but his temperature was 101°F (38 3°C) and he was suspected of having acute anterior poliomyelitis The cerebrospinal fluid on 9th, 11th and 13th August showed the following characters

	9th August	11th August	13th August
Cells/c mm	13	228	60
Lymphocytes	All	—	All
Protein/100 ml	25	60	60
Chlorides/100 ml	680	680	710
Culture	Neg	—	—

On 9th August the leucocyte count of the blood was 5,200 per c mm with 79 per cent of the cells polymorphonuclear

A specimen of serum taken on 8th August gave a weak reaction with *L icterohaemorrhagiae* at a dilution of 1/30, but was negative in higher dilutions and was negative with *L canicola* On these findings, and without any clear neurological signs, a diagnosis of non-paralytic poliomyelitis was made However, a specimen of blood taken on 20th August agglutinated *L icterohaemorrhagiae* to 1/1,000 and did not agglutinate *L canicola* Thus the diagnosis was made clear

CASES PRESENTING UNUSUAL FORMS

In a small number of cases abdominal signs and symptoms predominate, and may be sufficiently misleading to cause laparotomy to be carried out Ball (1933) and Herringman and Phillips (1947) report instances of patients operated on mistakenly as cases of cholecystitis, and the latter and Boquien, Hervouet, Dauphin and Verdier (1951) record two others in which appendicectomy was performed The following three cases *illustrate occasions when diagnosis was particularly* difficult

Case No 1—A boy aged 15 was admitted to hospital with pyrexia, abdominal pain, headache, backache, pains in the legs, vomiting and general malaise Since the abdominal pain settled in the right iliac fossa, a provisional diagnosis of acute appendicitis was made When he was anaesthetised for operation he had a severe epistaxis, and the operation was abandoned after an abdominal incision had been made Next day his serum agglutinated *L icterohaemorrhagiae* to 1/100, and a month later to 1/300 A fortnight later the brother of this patient was

taken ill with the meningeal form, and his case is described in the previous section *

CASE NO 2—In August 1948 we investigated an atypical case of a man of 26 years who took a cycling holiday in France from Dieppe to the Rhône valley, and bathed in and drank water from rivers and streams as a matter of principle—as it were to get a full taste of the country he was visiting On return to England he became ill with raised temperature, abdominal pains and a rash on the abdomen He had distinct headache, but no more definite evidence of meningeal irritation Typhus fever was suspected but the Weil Felix test was negative The agglutination test with *L icterohaemorrhagiae* however was positive to a dilution of 1/3 000, and with *L canicola* to 1/100

CASE NO 3—Another atypical case (Kernohan, 1956) was that of a man aged 58 whose illness commenced with shivering, pains in the limbs, vomiting and diarrhoea, which lasted for 3 days On the fifteenth day of illness he became very garrulous, noisy and confused This acute delirium lasted 36 hours and then passed off The cerebrospinal fluid was normal on examination, and there was no jaundice at any time His serum was found to have a titre of 1/1,000 for *L icterohaemorrhagiae*

Reference has already been made to the cases without localizing signs and symptoms—those in which the illness consists of only sufficient malaise, headache, muscular pains and temperature to cause an agglutination test to be done if circumstances or occupation suggest it These mild cases form a link with the subclinical infections revealed in sewermen and in rat catchers mentioned on p 101

A renal form of the disease has been recognized, in which there [illegible]

others

CONVALESCENCE

In all forms of leptospirosis convalescence is protracted, and

* We are indebted to Dr I B Morris for the specimens of serum and for clinical notes and pathological reports of these two cases

may last for several weeks or even for 3 to 6 months We have no records of our own cases, but the period of incapacity for which disablement pay was claimed by 67 patients in Australia (Table XVIII) was given by Stiles and Sawyer (1942) The

TABLE XVIII

DISABILITY CLAIMED IN AUSTRALIA FOR LEPTOSPIRAL INFECTION

(after Stiles and Sawyer, 1942)

Period of Incapacity	*No of Cases*
1 2 weeks	7
2–3 ,,	15
3–4 ,,	15
4–5 ,	9
5–7 ,	2
7 8 ,	4
10 ,	1
12 ,	1
24 ,,	1
> 26 ,	1
> 36 ,	1

infecting serotypes were not named, but were probably chiefly *L australis A* and *L australis B*

CASE MORTALITY

Most forms of leptospirosis show only a negligible or very small number of fatal infections, but a few serotypes produce a much more severe disease which carries a relatively high death rate Infections with *L icterohaemorrhagiae*, *L andaman A*, *L pyrogenes* and *L bataviae* (in Indonesia) are those most likely to prove lethal

The proportion of deaths recorded in any series of cases of Weil's disease is greatly affected by the number of mild infections which have been included This in turn depends on the clinical acumen of medical practitioners and on the laboratory facilities available Thus case mortality rates in Weil's disease recorded in different countries or at different times have varied from 5 to 30 per cent

PRESENCE OF JAUNDICE

It is well known that the occurrence of jaundice and its intensity give good indications of the severity of the illness

Fromme (1930) quoted 2 cases of 100 and 103 days respectively [illegible]

who observed leptospires in the urine of a man 11 months after recovery from an infection with *L australis B.*

SECOND ATTACKS

No authenticated second attack of leptospirosis due to the [illegible]

diagnosis was confirmed serologically at the time of each of the two attacks, which were separated by periods of two to three years in each patient In four other cases the diagnoses of the first attacks were made on clinical grounds, but the patients sera contained antibodies for two unrelated serotypes at the time of the second illness The serotypes involved were in most cases, *L pomona* and *L hyos*

In the case described by Doherty (1956) the strain causing the first attack was isolated and identified as the Kremastos type The patient was re-admitted to hospital 23 days after his discharge with a second acute attack of fever The causative organism was isolated on this occasion also and proved to be *L hyos* Two strains were also isolated in Malaya from a patient whose second attack occurred about three months after the first The strains were identified respectively as *L schüffneri* and *L bataviae* (Broom unpublished)

CONCURRENT INFECTIONS

A simultaneous infection with *L hyos* and the Szwajizak type (later named *L mini*) was thought to be the most likely explanation of curious reactions obtained with a culture isolated from a patient in Australia (Queensland 1955) When first examined the culture was agglutinated to high titre only by an antiserum to *L. hyos* During the next four months the strain was subcultured nine times, and it was then found to react negatively with *L hyos* antiserum Further investigation showed that the

observations were made on cases which occurred in Malaya among members of the Armed Forces Atkins, Broom, Freezer and Harvey (unpublished) examined 50 men, all coal miners who had suffered an attack of Weil's disease at various times from 1 to 15 years earlier No evidence was obtained of residual damage to either the liver or kidneys

As was mentioned earlier (p 107) lesions affecting the eyes may occur months after recovery from the acute attack but only very rarely, as in a case described by Strobel (1952) is there *permanent impairment of vision*

Murgatroyd (1937) described a unique case of a patient who after the acute stage of a moderately severe attack of Weil's disease, continued with irregular pyrexia for four months Severe meningitis then developed, and leptospires were isolated from the cerebrospinal fluid 25 weeks after the illness began Leptospires were also present in the urine in the thirty-third week and the patient finally recovered after 8 months of illness

Evidence that headaches of a recurrent migrainous type may be the result of leptospiral infection was put forward by Atkins (1955) He found that 24 out of 50 coal miners who had recovered from Weil's disease were affected with migraine and that only 4 of these men had had headaches before the illness By contrast only 6 out of 50 miners who had not suffered from Weil's disease complained of similar headaches Dr E *Montuschi and Dr T St M Norris (personal communications)* have told us of 3 other patients who complained of recurrent headaches after Weil's disease or canicola fever Atkins pointed out that specific infection is not mentioned by well known writers as a cause of migraine He suggested that further information might be obtained by studying the after histories of patients who had recovered from other types of meningeal infection

CARRIER STATE

There is no evidence that a carrier state of indefinite duration ever develops after leptospirosis in man, but cases are on record in which excretion of leptospires in the urine had continued well beyond the normal period of 4 to 6 weeks from the onset of disease As regards *L icterohaemorrhagiae*, Uhlenhuth and

Fromme (1930) quoted 2 cases of 100 and 103 days respectively and, in the case described by Murgatroyd mentioned above, this serotype was still present in the urine 33 weeks after the illness began A still longer period was reported by Johnson (1950) who observed leptospires in the urine of a man 11 months after recovery from an infection with *L australis B.*

SECOND ATTACKS

No authenticated second attack of leptospirosis due to the *same serotype has been reported*, but there have been a certain number of instances of two attacks caused by different serotypes. Thus Gsell (1954) recorded four instances in which the diagnosis was confirmed serologically at the time of each of the two attacks, which were separated by periods of two to three years in each patient. In four other cases the diagnoses of the first attacks were made on clinical grounds, but the patients' sera contained antibodies for two unrelated serotypes at the time of the second illness The serotypes involved were, in most cases, *L pomona* and *L hyos*

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strain reacted to low titres with antisera for *L medanensis* and the Kremastos type, and to high titre with Szwajizak antiserum

As a result of this discovery, subcultures were made from the original culture, which still contained a few viable leptospires This subculture reacted to high titre with Szwajizak antiserum but gave no reaction with *L hyos* antiserum From these findings the assumption was made that the patient had been infected by two serotypes at the same time *L hyos* was predominant in the early subcultures, but it was subsequently overgrown and finally replaced by the Szwajizak strain

Wiesmann and Suter (1956) reported a simultaneous infection with *L pomona* and *L hyos* The patient was employed in a piggery where, in spite of advice to the contrary, he worked barefoot He was taken ill with a severe attack of swineherd's disease only ten days after he began work Leptospires were isolated in culture from samples of blood and cerebrospinal fluid taken on the sixth day of illness The strain from the cerebrospinal fluid was identified as *L pomona*, but the culture from the blood reacted partly as *L pomona* and partly as *L hyos* By growing the strain in separate cultures containing antisera for *L pomona* and *L hyos* respectively, it proved possible to obtain pure cultures of the two serotypes, and to determine their identities

Alexander, Evans, Toussaint, Marchwicki and McCrumb (to be published) referred to four cases of double infection by *L icterohaemorrhagiae* and *L bataviae* in Puerto Rico

CHAPTER IX

EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF MILDER LEPTOSPIROSIS

As Exemplified by Leptospirosis Canicolaris (Canicola Fever)

HISTORY

Infection of dogs with *L canicola* was recognized by Klarenbeek and Schuffner (1933), but the first two human cases were recorded by Dhont, Klarenbeek, Schüffner and Voet (1934) in the Netherlands One patient was a boy aged 16 who had been in contact with a fox-terrier The illness was mild with transient jaundice of the sclerae , both the boy and the dog showed clear serological evidence of infection by *L canicola* The second patient was a man aged 51, and a strain of *L canicola* was isolated from him A further nine infections were found in the Netherlands by 1937 (Schüffner, Kotter and Schultsz, 1935 , Schüffner and Walch-Sorgdrager, 1937 Roos Walch-Sorgdrager and Schuffner, 1937) In 1938 Brammer, Borg-Petersen and Scheel-Thomsen reported an infection in Denmark, and since then cases have been recorded from most parts of the world

EPIDEMIOLOGY

CARRIER HOSTS

DOGS —So far as is known human beings are most often infected by direct or more remote contact with dog urine In the majority of cases the human patient has had more or less close association with a dog which is either suffering from an acute infection with *L canicola*, or is a healthy carrier Rosenberg (1951) reviewed 200 human cases and found that mention of the presence or absence of a likely source of infection was made in 131 There was a record of close

infected from the same pool in Germany In Westphalia 8 children developed the disease after bathing in a muddy pool along with 2 dogs which had positive agglutinin titres for *L canicola* (Primavesi 1951) Campbell *et al* (1950) found a similar source of infection in England

Larger groups of people have also been infected in this way —for instance 26 children from a stream in Georgia U S A (Williams *et al* 1953) Another group comprised 24 persons who probably contracted the disease in an open air swimming pool in Wyoming U S A The cause of the illness was not suspected until 11 years after the time of the epidemic in 1949 It was then possible to obtain samples of serum from only 9 but all contained agglutinins for *L canicola* (Cockburn Vavra, Spencer Dann Peterson and Reinhard 1954) A total of at least 114 persons were infected by bathing in a village in the Fukuoka Prefecture Japan in 1953 (Misao Hiroyoshi Katsuta Nishihara Kobayashi Kuwashima and Aso 1956)

Field Work

Of 32 cases of leptospirosis among workers in rice fields near Modena North Italy 3 were found by Berengo and Bussinello (1952) to be due to *L canicola* In Japan 43 cases of canicola fever were diagnosed among field workers engaged in trans planting rice during a wet period when streams were overflowing into the fields (Misao *et al* 1954)

Other Methods of Infection

Professional infection of veterinary workers has been recorded by Meyer Stewart Anderson and Eddie (1938 a & b) and Joe and Sangster (1951) and infection of a woman by a dog bite was considered probable by Lereboullet *et al* (1949)

Sex and Age Incidence

The distribution of cases between the sexes has been found to be approximately equal Rosenberg (1951) reviewed 200 cases and found that 56 per cent were males The age dis tribution of infections shows cases at all ages but they are rarer in infants and small children than might be expected from the possible risk fr[illegible] g on th[illegible] see Tables XI and XII)

SEASONAL INCIDENCE

In most countries the disease occurs chiefly in the second half of the year, Borg-Petersen (1944 a) found in Denmark that during the years 1934 43 the onset in 33 out of 47 infections was in the months of October, November or December Rosenberg (1951) found that in three-quarters of 200 cases collated from world literature the illness began between July and December Broom (1951 a) found for England and Wales that 75 per cent of 70 cases began during the months June to November in the period 1947-50

MORBID ANATOMY

Complete recovery from canicola fever is the rule, and only two well-authenticated fatal cases have been traced The first of these occurred in the Netherlands in 1947 and was described by Wolff, van Dam and Minkenhof (1951) The patient's blood urea was 535 mg per 100 ml on the day before death At postmortem the body was icteric and there were petechial haemorrhages in the pericardium and pleurae The kidneys showed the type of lesions described in fatal infections by *L icterohaemorrhagiae* There was swelling of the epithelium of the proximal convoluted tubules, many of the distal convoluted tubules were blocked with granular brown casts and there were scattered collections of lymphocytes, plasma cells, macrophages and polymorphonuclear leucocytes throughout the interstitial tissue

The second fatal infection was recorded by Weetch *et al* (1949) This patient was a woman aged 35 who had had hyperpiesis and albuminuria during her first pregnancy sixteen years before her death, and severe headaches during four later pregnancies The fatal illness lasted 24 days, and in the early stages albuminuria and tenderness in the loins were the only signs of renal involvement At that time the non-protein nitrogen of the whole blood was 45 mg per 100 ml In the last week before death there were oliguria, a non-protein nitrogen concentration of 284 mg, and finally uraemia and acidosis At postmortem the kidneys were enlarged and the cortex was pale and had small haemorrhages Histologically

there was chronic interstitial nephritis, with superadded acute interstitial and tubular nephritis It thus appears that as in infection by *L icterohaemorrhagiae* the factor which determines death or recovery is the degree of acute nephritis, and that pre-existing chronic nephritis increases the risk to life Two less well-authenticated instances of fatal infection by *L canicola* have been published—one in the U S A by Molner, Meyer and Raskin (1948), and the other in Viet-Nam by Kolochine-Erber, Brygoo, Cros and de Lajudie (1952)

CLINICAL PATHOLOGY

Blood

There is often a moderate degree of anaemia, the erythrocyte count falling towards 4 million per c mm , and the haemoglobin value being reduced proportionately In an unusually severe case which showed nephritis and anaemia the red corpuscles were 2 7 million (Lereboullet, Kolochine-Erber and Lambert, 1951 a) In the early stages the total leucocyte count may be normal or somewhat diminished Later, a leucocytosis of 10 to 15 thousand per c mm is common with a relative polymorph preponderance, but this is by no means invariable

In the majority of cases the concentration of urea is within normal limits, or only raised to 50 to 60 mg per 100 ml Of 63 cases of which we have records only 6 showed higher levels (92 to 160 mg) and in the two fatal cases mentioned above the concentration reached 284 mg and 535 mg The blood sedimentation rate is markedly increased from the first few days, and remains high during the illness and well into convalescence

Cerebrospinal Fluid

In the early stages the fluid may show no abnormalities, but by the fourth or fifth day the protein concentration is increased to 60 to 80 mg per 100 ml with a relative excess of globulin The concentration of glucose is within normal limits The cell count also rises, [illegible] reaching 2,000 or more per c mm , with either s[illegible] or [illegible] pre-dominating.

URINE

The urinary findings vary widely, depending on the extent to which the liver and kidneys are affected The urine may only show from time to time traces of albumin, it may be loaded with bile pigments, albumin and granular and hyaline casts, or the abnormal constituents may be present in any degree between these two extremes

MUSCLE

Inflammatory lesions similar to those found in infections by *L icterohaemorrhagiae* have been found in muscles by biopsy (Turrell and Hamburger, 1951)

SYMPTOMS AND COURSE OF ILLNESS

Canicola fever may be taken as representative of the milder forms of leptospirosis in which meningeal symptoms are a common feature In Table XX are set out the relative frequency of various symptoms in 127 cases which occurred in

TABLE XX

CANICOLA FEVER BRITISH ISLES 1949-55
SYMPTOMS IN 127 HUMAN CASES

	Per cent		Per cent
Headache	92	Albumin in urine	48
Meningitis	75	Casts in urine	19
Neck stiffness	71	Rash	18
Injection of eyes	49	Jaundice	19
Muscle tenderness	47	Haemorrhages	14

Great Britain from 1948 55 Since complete details are not available for every patient the frequencies have been expressed as percentages to facilitate comparison

As in other forms of leptospirosis the onset is usually sudden with shivering or rigors and intense headache The temperature rapidly mounts to 103°-104°F (39 5°-40°C) and the patient complains of photophobia, tenderness of muscles, nausea and vomiting Prostration is marked, often to a greater degree than the severity of the symptoms seems to warrant Profuse sweating often occurs early Signs of meningeal irritation are

common and may include mental confusion, vertigo, stiffness of the neck and Kernig's sign Weakness or even paresis of muscles may be present, giving rise to a suspicion of polio myelitis, the tenderness of muscles may cause an immobility which simulates paresis

On some occasions congestion of the lungs is marked simulating the early stages of pneumonia, or abdominal symptoms may predominate suggesting a diagnosis of enteric fever Myocarditis has occasionally been recorded and some degree of diminution of cardiac efficiency is not uncommon Relative bradycardia and a reduced blood pressure are usually present

Injection of the conjunctival vessels is found in about one half of the cases and is a useful diagnostic sign when present Rashes, either morbilliform, scarlatiniform or herpetic in type or, later in the illness, a rash resembling the rose spots of typhoid fever frequently appear, but these eruptions are often fleeting and may not be noticed unless they are specially looked for Jaundice is much less common than in Weil's disease, and even when present may show only as a subicteric tinge

The kidneys are generally affected, though the degree of involvement varies very widely The evidence of damage may range from occasional traces of albuminuria to the signs of acute nephritis Thus Bukh (1940) and Audoly (1948) described non fatal cases in which the blood urea reached 280 mg and 576 mg per 100 ml respectively In the first two known fatal cases, reported above, severe renal damage was present

The initial fever may last for only a day or two or may continue for a week or more before the temperature falls by lysis In either case a recurrence of pyrexia frequently follows the afebrile period, and further relapses though uncommon have been reported When the primary attack is short, acute meningeal symptoms often develop with the secondary rise of temperature, and this is the form of disease most frequently confused with poliomyelitis

Signs of ocular involvement occur as in Weil's disease (see p 107) Williams (1956) described a case complicated by homonymous hemianopia in which the condition was unchanged after nine months Although encephalitis has not often been recorded in canicola fever it has been found in infection by

some of the other milder serotypes (Mach, 1944) and examples are given under *L hyos* and *L pomona*

Middleton (1955) described paralysis of the right serratus anterior muscle and weakness of the right side of the diaphragm and of the left thumb, which developed during convalescence from canicola fever The lesions were considered to be in the cervical spinal cord or its roots or nerves, recovery was complete after eighteen months Mortensen (1940) described a case of flaccid paralysis of the legs due to infection by *L sejroe* and quoted four other similar lesions of the spinal cord

The severe and intractable headache is an outstanding and most distressing symptom of canicola fever It is quite unaffected by drugs, but marked relief is often obtained by removal of cerebrospinal fluid even when the tension is not increased Partial loss of hair may occur persisting for different lengths of time before regrowth

Convalescence

The length of convalescence is variable and the illness often extends to two, three or four months Physical weakness is the most tedious feature of this stage, and late signs of ocular involvement may occur

ILLUSTRATIVE CASE HISTORIES

(from Laurent Norris Starks Broom and Alston 1948)

Case 1

A boy aged 19 was admitted to hospital on 15th September 1947, as a case of cerebrospinal meningitis He had been taken ill suddenly on 11th September with frontal headache, fever and sweating Next day his eyes were red and the neck stiff On the fifth day a pink blotchy rash appeared on the face and chest The bowels had been confined four days and no drugs had been taken

On Admission—Fifth day of illness Temperature 100 8°F (38 8°C) pulse rate 68, respiration rate 22 per min The patient was a well-nourished young man, conscious, alert and co-operative The palpebral and bulbar conjunctivae were

intensely injected and red, with little discharge and pronounced photophobia There was a pink discrete macular rash chiefly on the chest, abdomen and back, *and a little less on the face* and extremities The rash was morbilliform but lighter in colour than that of measles, and not irritating He had no cough, no Koplik's spots and no redness or ulceration of the buccal mucosa There was no enlargement of the lymph glands or spleen and no jaundice Clinical examination of heart, lungs and abdomen revealed nothing abnormal Blood pressure 130/80 mm Hg , urine, sp gr 1020, no abnormal constituents There was no urethral discharge and no sore on the genitals There was some stiffness of the neck on flexion but Kernig's sign was negative Cranial nerves, fundi and ears were normal There was no loss of power or sensation and all the reflexes were normal Lumbar puncture fluid clear and *colourless with less than 4 cells per c mm , protein* 30 mg per 100 ml , and no organisms

Subsequent Course—On 19th September (eighth day) his temperature was 101 4°F (38 6°C), he was drowsy, his neck was more stiff and Kernig's sign was positive Lumbar puncture now gave a faintly opalescent spinal fluid with 290 cells per c mm (mononuclears 50 per cent), protein 30 mg per 100 ml , chlorides 680 mg , no organisms and sterile on culture White cell count 6,800 per c mm (polymorphs 76 5 per cent) Blood culture (twice) Widal test, Wassermann and Kahn tests and Paul-Bunnell test were all negative X ray films of the nasal sinuses showed no evidence of infection

On 20th September (ninth day) his temperature was normal and it remained normal afterwards The rash faded rapidly after that date without leaving any staining of the skin On 3rd October (twenty-second day) his eyes were normal, all traces of meningitic signs had disappeared, and no abnormal neurological signs could be detected He was discharged home quite well on 20th October, the thirty-ninth day after the illness began

By a series of agglutination tests of blood taken on the twenty-fourth, fiftieth and hundredth days after the onset a diagnosis of infection by *L canicola* was established

The patient had kept a puppy, six months old, just before his illness, but so far as he knew the dog was healthy

Unfortunately this dog was accidently killed before the patient's infection was diagnosed

CASE 2

A woman aged 40 was admitted to hospital on 1st October 1947, with the following history On 23rd September she had headache and malaise and in the evening suffered from an attack of shivering Next day the symptoms were worse and there was aching pains in the limbs The patient collapsed on getting out of bed, and her temperature was found to be 105°F (40 6°C) when she was seen by her doctor later in the day On the third day meningitis was suspected and a course of sulphathiazole treatment begun On the fourth day vomiting became troublesome, and a mistiness of vision developed in both eyes and lasted for 48 hours The patient was unable to see objects directly ahead but could see things 'out of the corner of her eyes fairly clearly On the fifth day spots, similar in appearance and distribution to erythema nodosum, appeared on both legs, but they cleared in about 2 days By the eighth day it was evident that the condition was not responding to sulphonamides and the patient was sent to hospital for further investigation

On Admission—Ninth day of illness The patient still complained of severe occipital headache and pain at the back of her neck There were now no pains in the limbs and no further nausea or vomiting Micturition was normal, but she was severely constipated She looked ill and was flushed and perspiring freely Temperature 101°F (38 3°C), pulse rate 86, respiration rate 24 per min Herpes labialis was present There was no rash or lymphadenopathy There was slight but definite neck rigidity and the pain in her neck was much increased by flexing the cervical spine Kernig's and Brudzinski's signs were both negative No other abnormality in the central nervous system was noted There was neither conjunctivitis nor suffusion of the eyes The optic fundi were normal, and rough tests showed no alteration in the visual fields Blood pressure 120/80 mm Hg The cardiovascular system was normal apart from a soft basal systolic bruit The urine was sterile on culture, contained a trace of albumin, and the centrifuge deposit showed a few red blood corpuscles and

some leucocytes Blood count 3,700,000 red cells per c mm., 6,000 white cells per c mm (polymorphs 69 per cent), hæmoglobin 76 per cent Lumbar puncture fluid clear, initial pressure 120 mm water, 48 cells per c mm, protein 20 mg per 100 ml, glucose 57 mg, culture sterile

Subsequent Course—On 3rd October (tenth day) her headache was still severe and she was perspiring freely, no change in physical signs Lumbar puncture 233 cells per c mm (lymphocytes 95 per cent), protein 20 mg per 100 ml, culture sterile Blood urea 40 5 mg per 100 ml, Paul Bunnell reaction negative

Leptospirosis was considered as a diagnosis and the agglutination reaction was found to be positive for *L canicola* and for *L icterohaemorrhagiae*, though to a lower titre During the next few days the patient gradually improved, and by the fifteenth day her temperature was normal and the symptoms had disappeared

On 14th October (twenty first day) the headache recurred though less severely, and the temperature rose to 100 6°F (38 1°C) with early morning remissions This relapse lasted for ten days during which time the symptoms improved and the temperature fell slowly Recovery thereafter was uneventful and the patient was discharged from hospital on the fifty first day For the next fortnight the patient complained of weakness of the leg muscles, affecting particularly dorsiflexion of the feet Her hair, which had begun to fall out while she was in hospital was getting thinner, and the patient feared she might be going bald When she was seen again 3 months later however the hair had started to grow again and the muscular weakness had cleared up

For six months before her illness this patient had owned a dog which was in bad health A specimen of the dog s serum agglutinated *L canicola* to a titre of 1/3,000 and *L icterohaemorrhagiae* to 1/30 The dog was destroyed before any other *investigations could be made*, but there seems little doubt that it was the source of the patient's infection

CHAPTER X

EPIDEMIOLOGY, PATHOLOGY AND CLINICAL ASPECTS OF LEPTOSPIROSIS DUE TO OTHER SEROTYPES

L naam	*L pyrogenes*
L mankarso	*L australis B*
L javanica	*L sentot*
L poi	*L autumnalis*
L sarmin	*L bangkinang*
L schüffneri	*L djasiman*
L benjamin	*L australis A*
L ballum	*L muenchen*

L pomona

In this Chapter and in Chapter XI, serotypes which cause human infections are considered in the same order as they are placed in Table II The Table also includes *L icterohaemorrhagiae* and *L canicola* which are dealt with in Chapters VIII and IX respectively *L cynopteri* is referred to in Chapter XVII since it is not known to have infected human beings Chapter XI also includes an account of serotypes causing human infection which have not yet been fully compared with other known serotypes

L naam

A single strain, serologically related to *L icterohaemorrhagiae* and to *L mankarso* in the Icterohaemorrhagiae group, isolated by Wolff from a fatal infection of a Javanese labourer in 1936 (Walch-Sorgdrager, Bohlander, Schuffner and Wolff, 1940)

L mankarso

A serotype related to *L icterohaemorrhagiae* and *L naam* in

the *Icterohaemorrhagiae group* and first isolated from a man in Sumatra in 1938 (Wolff, 1953 a) Other strains were isolated from a man in Malaya and from a rat (*Rattus whiteheadi*) in North Borneo (Alexander *et al*, 1955)

L javanica

This serotype is related serologically to *L poi* and *L celledoni* Eleven strains were first isolated from field rats in Java (Esseveld and Mochtar, 1938) *In Indonesia it has been isolated from Herpestes javanicus, R rattus diardi, R brevicaudatus, R rattus jalorensis, R concolor, R bartelsi* and *R norvegicus*, and also from dogs and cats (Collier, 1948 a) Serological evidence of a few human infections has been found and the disease is probably of a mild type For instance, two human infections *by leptospires of the Javanica serogroup (not more fully* identified) were found in Malaya by Fairburn and Semple (1956) A strain closely related to *L javanica* caused a mild febrile illness in a laboratory worker in London (Broom and Norris, 1957) The serotype shows little pathogenicity *for* guineapigs

L poi

(Syn *L sorex*)

Mino (1942 a) isolated a new serotype of leptospires, which he named *L poi* from a rice worker near *Vercelli in Northern* Italy It was considered to be related antigenically to *L icterohaemorrhagiae* and *L ballum*, but Wolff and Broom (1954) found it closely related to *L javanica*, only slightly to *L icterohaemorrhagiae* and not to *L ballum* Borg Petersen (1949) found serological evidence of infection by *L poi in two cases* of leptospirosis in Denmark during 1945 and 1946 Berengo and Bussinello (1952) found an infection near Modena and Austoni (1953) stated that other cases had been found in different districts in Italy One case occurred in Malaya (Fairburn and Semple, *1956*), and Kmety *(1955 a) isolated* from a shrew (*Sorex araneus*) a strain which he named *L sorex* and later (1955 b) showed to be identical with *L poi*

L sarmin

This serotype is represented by only one strain isolated from a man in Indonesia by Kotter in 1930 (Kotter, 1939)

L schuffneri

(Syn strain 90 C)

This serotype was first isolated from bats in Indonesia in 1938 by Collier and Mochtar (1939 a) Serological evidence of infection was found by these observers in a dog in Java, and by Das Gupta (1939 c) in a human case in the Andaman Islands In Malaya three human strains were identified by Broom (unpublished) and strains were isolated from *R rajah* by Alexander *et al* (1955) and from *R exulans* by Gordon Smith and Broom (to be published) The illness produced in human beings is mild

L benjamin

This serotype (which is related to *L schuffneri*) was first isolated from a man in Sumatra in 1937 (Walch-Sorgdrager *et al* 1940) It has been found by Gordon Smith and Broom (to be published) in *R exulans* in Malaya Strains of the Mukingilwa type isolated from human cases in the Belgian Congo by van Riel (1946) were stated by him to represent the incomplete biotype (A) The illness produced in man is mild

L ballum

In October 1943 Borg Petersen (1944 c) isolated from the urine of a mouse caught near Ballum South Jutland, a new serotype which he named *L ballum* The mouse was considered to be *Mus musculus spicilegus* but from the evidence put forward by Mohr (1950) and summarized on p 159 it was most probably *M musculus musculus*

The first human infected by this serotype was a laboratory worker in Amsterdam (Borst *et al*, 1948, Wolff *et al*, 1949) The illness began acutely with high fever and severe headache, there were joint pains, conjunctival injection herpes labialis and severe dizziness The temperature fell by lysis after five days

and convalescence took three weeks Successive specimens of the patient's serum showed increasing agglutinin titres against *L ballum* up to 1/3,000 on the sixty-second day after onset, and gave little or no reaction with a wide range of other serotypes The patient remembered that she had been slightly scratched on the finger by a laboratory mouse 9 days before the illness, the scratch had been immediately treated with ethanol When the mouse was examined after the patient's illness its serum agglutinated *L ballum* to 1/300, and *L ballum* was being excreted in the urine Of apparently healthy adult mice bred in the same laboratory, 28 out of 30 were found to be excreting leptospires in the urine intermittently during a period of more than a year without affecting health or breeding *L ballum* was isolated from the kidneys of one and was also cultured from the only two mice which were not excreting it. By contrast only 1 out of 15 young mice from the same stock was infected Five other stocks of mice in the Netherlands were examined, four stocks were clean, but 63 out of 80 animals in the fifth were excreting leptospires

Other countries in which carrier hosts have been recognized are

Czechoslovakia Pigs (Kmety, Plesko and Chylo, 1956)
Portugal *Apodemus sylvaticus* and *R norvegicus* (Fraga de Azevedo, Valente and Queiros, 1951)
Canada (British Columbia) *R norvegicus* (Humphreys Campbell and Smith, 1953)
U S A Laboratory and house mice (Yager, 1953), house mice and an opossum (Yager, Gochenour, Alexander and Wetmore, 1953)

One human infection by *L ballum* was diagnosed in a farm worker in Yugoslavia (Falisevac, 1951), and two others in Puerto Rico (Yager, 1953) Picard (1954) isolated leptospires from workers in rice fields situated in the delta of the river Rhône, and these strains were identified by Kolochine Erber (1953) as *L ballum* Picard considered that about 80 per cent of the infections contracted in these rice fields were caused by this serotype Covaleda, Pumarola and Cantarell (1953) found serological evidence of infection with *L ballum* among ricefield workers on the delta of the river Ebro in Spain

Babudieri (1955) compared the serological characters of human strains isolated in Italy with those of the type strain He found that the Danish strain represented the incomplete biotype *L ballum* (A), and the Italian strains were the complete biotype (AB)

Geographical distribution of human infections

EUROPE

FRANCE Picard (1954)
NETHERLANDS Wolff *et al.* (1949)
SPAIN Covaleda *et al* (1953)
YUGOSLAVIA Taliseyac (1951)

AMERICA

PUERTO RICO Yager (1953)

L pyrogenes
(Syn Salinem strain, *L febrilis*, *L okinawa*)

This serotype was first recognized in Indonesia by Vervoort (1923) and then by Kouwenaar (1924) It has been known as the Salinem strain, *L febrilis* (Bonne, 1924 b) and as *L okinawa* in Japan (Kitaoka, 1951) *L pyrogenes* is closely related to *L australis B* but the latter is not the incomplete biotype of *L pyrogenes* as was believed at one time Yamamoto (1948) showed that the two serotypes share a common antigenic fraction but that each possesses specific fractions in addition These findings were confirmed by Wolff and Broom (1954) *L pyrogenes* has been found in Java in *R brevicaudatus* (Esseveld and Mochtar, 1938), and in 'house rats' in Japan (Kitaoka, 1951)

The disease may be a non-fatal anicteric illness with fever, conjunctival congestion and sometimes meningitis, or it may be severe and fatal (Kouwenaar, 1924) It is nearly always contracted during work in the fields The serotype is pathogenic to guineapigs, producing jaundice

Geographical distribution of human infections

EUROPE

ITALY Austoni (1953)

ASIA

INDONESIA Vervoort (1923), Kouwenaar (1926)
JAPAN Kitaoka (1951)
MALAYA Fletcher (1928), Trimble (1954)
OKINAWA Kitaoka (1951)

L australis B
(Syn Zanoni strain)

HISTORY —In 1936, Cotter gave an account of a series of outbreaks (chiefly among sugarcane cutters) of an illness regarded clinically as Weil's disease which had been seen in the coastal area of North Queensland since 1933 In 1937 Lumley reported serological studies on strains of leptospires which had been isolated from some of these cases during the previous years He found that there were two contrasting agglutinin groups among 24 human sera, and two contrasting agglutinogens among 19 strains Within each group the strains approximated very closely to one another serologically, and he named the groups *L australis A* and *L australis B* Lumley found that these two serotypes differed antigenically from *L icterohaemorrhagiae*, *L pomona*, *L autumnalis* and *L canicola* The strain names of Ballico and Zanoni were also applied to the two serotypes respectively Schuffner (unpublished work quoted by van Thiel, 1948 a) considered *L australis B* to be the incomplete biotype of *L pyrogenes* However, Yamamoto (1948) showed that they were separate though related serotypes and the same finding was reported by Wolff and Broom (1954)

EPIDEMIOLOGY —Infection with *L australis B*, in the same way as *L australis A*, is associated with growing and cutting sugar cane, in the narrow well watered coastal belt of North Queensland between Ingham and Cooktown, but *L australis B* also infects urban dwellers in the same territory Derrick (1956) reported that of 219 leptospiral infections in North Queensland from 1951-54, 58 (26 per cent) were due to *L australis B* Excellent accounts of the epidemiology of the disease in the cane fields are given by Cotter (1936), Johnson (1959), Doherty, Emanuel and Moore (1956), Derrick (1936) and these are summarized on p 287

CLINICAL ASPECTS —The clinical features of the illness are

those of other comparatively mild forms of leptospirosis The illness often begins suddenly with shivering fever and comparatively slow pulse rate Headache, lassitude, loss of appetite, conjunctivitis and limb pains are usual Upper abdominal pain occurs in about half the patients, jaundice is seen only in a minority and is rarely severe Enlargement of axillary glands is often present Johnson (1950) recorded one death among 29 patients infected by *L australis B*

Infection by *L australis B* has also been found in men in North Italy (Mino, 1942 b) and in Malaya (Fairburn and Semple, 1956) Kmety (1954) reported infections in Czechoslovakia by *L australis A* and *L australis B*, but later stated that the strains concerned were *L pomona* Puntigam and Till (1950) and Falisevac (1951) reported human infections by *L australis* in Austria and Yugoslavia respectively, but they did not state whether *L australis A* or *L australis B* was involved

Geographical distribution of human infections

EUROPE

ITALY Mino (1942 b)

ASIA

MALAYA Fairburn and Semple (1956)

AMERICA

BRAZIL Correa, Amato Neto Veronesi and Brandão (1954)

AUSTRALASIA

QUEENSLAND Cotter (1936) Lumley (1937) Johnson (1950)

L sentot

This serotype was first isolated from a man in Sumatra in 1937 (Schüffner, Gispen and Bohlander 1939) Another strain isolated from a patient in Malaya was identified as *L sentot* by Broom (unpublished)

L autumnalis

(Syn *L akiyami A L hebdomadis A*, Rachmat strain)

HISTORY—A disease similar to nanukayami from which

Ido *et al* (1918) isolated *L hebdomadis* in the Province of Fukuoka, Japan (p 156), occurred in Shizuoka Province where it was known as autumn fever or akiyami Kitamura and Hara (quoted by Koshina, Shiozawa and Kitayama, 1925) found that autumn fever was leptospirosis, but that the blood of some patients was more virulent to guineapigs than that of others

Koshina *et al* completed the investigation of autumn fever by studying an outbreak in the region of the Oi River where many cases had occurred annually They obtained, by guineapig inoculation, cultures of leptospires from 12 patients, 3 of them were more virulent for guineapigs than the others The more virulent strains—which they called Akiyami A type—caused jaundice and haemorrhages in the tissues, and leptospires were more numerous in the liver Subsequent passage was always successful, whereas passage of the less virulent strains (Akiyami B type) failed at the second to the fifth transfer

These workers showed by Pfeiffer's reaction and by agglutination and protection tests that Akiyami B type was identical with *L hebdomadis*, and that Akiyami A type was serologically distinct from *L hebdomadis* and *L icterohaemorrhagiae* Stefanopoulo and Hosoya (1928) repeated some of these comparisons, and confirmed that Akiyami A type was as pathogenic as *L icterohaemorrhagiae* for guineapigs

Abe (1934) proposed the name *L autumnalis* for the Akiyami A type The Rachmat strain, isolated in Indonesia by Baermann (1923) and by Wolff (1925), was shown by Gispen and Schuffner (1939 a) to be the incomplete biotype (A) of *L autumnalis*

Human infections were reported from Malaya by Fletcher (1928) and Trimble (1954), from the Andaman Islands by Brown (1928), from Sumatra by Esseveld (1938), from Thailand by Sundharagiati and Buspavanich (1951), from North Borneo by Wisseman, Traub, Gochenour, Smadel and Lancaster (1955) and from French Indo-China by Vaucel (1938) Gsell (1952) mentioned that Wiesmann had twice found serological evidence of infection in Europe—once in a fatal case in Münsterlingen, Germany, and once in a patient in Strassburg, France

In the U S A, Gochenour, Smadel, Jackson, Evans and Yager (1952) found by retrospective serological tests that an illness known as Fort Bragg fever or pretibial fever was due to *L autumnalis* During each of the summers of 1942, 1943

and 1944 about 40 cases were diagnosed [illegible] North Carolina, U S A The disease was [illegible] 5 days' duration and exhibited headache, [illegible] megaly, distinctively, there was an erythematous [illegible] limited to the pretibial areas of both legs [illegible] spires was isolated from a hamster infected [illegible] representing the 365th passage of the infecting [illegible] guineapigs, eggs and hamsters, and was [illegible] *autumnalis* Experiments made on human [illegible] chimpanzees showed that the strain isolated from [illegible] was the original infective agent

EPIDEMIOLOGY AND CLINICAL ASPECTS —Koshina [illegible] found that in one province of Japan *L autumnalis* [illegible] by the field mouse *Microtus montebelloi*, and [illegible] the carrier to be *Apodemus speciosus* in [illegible] Alexander *et al* (1955) found the serotype in [illegible] Thailand Autumn fever is practically limited [illegible] between August and October (as is seven-day [illegible] infection is chiefly among persons engaged in [illegible] and most of the patients are under 20 years of [illegible] picture is indistinguishable from infection [illegible] which is described on p 158

Geographical distribution of human infection [illegible]

EUROPE

FRANCE Gsell (1952)
GERMANY Gsell (1952)

ASIA

ANDAMAN ISLANDS Brown (1928)
FRENCH INDO CHINA Vaucel (1938)
INDONESIA Baermann (1923) Wolff (19[illegible]
Gispen and Schuffner (1939 a)
JAPAN Koshina *et al* (1925)
MALAYA Fletcher (1928) Trimble (1954
NORTH BORNEO Wissemann *et al* (1955)
THAILAND Sundharagiati and Buspavani

AMERICA

U S A Gochenour *et al* (1952)

L bangkinang

This serotype is related to *L autumnalis*, and was isolated in 1929 from a man in Indonesia (Slot and van der Walle, 1932), 7 human infections were found in soldiers in Malaya (Trimble, 1954) Clinically it causes a mild disease

L djasiman

The original strain was isolated in 1937 from a man in Indonesia (Kotter, 1939) Strains were also isolated by Gordon Smith and Broom (to be published) from *Rattus bowersi* in Malaya

L australis A

(Syn Ballico strain, *L akiyama C*, *L hebdomadis C*, *L tenryuensis*)

HISTORY —The differentiation of *L australis A* and *L australis B* was made by Lumley (1937) and has already been described (p 138) In 1938, strains first named Akiyami C and later *L hebdomadis C* and *L tenryuensis* were isolated from patients living along the course of the Tenryu River and in other areas in Japan and were found to be identical with *L australis A* (Kitaoka, 1951) Alicata (1949) had suggestive evidence of human infection by *L australis A* in Samoa Human cases of infection by this serotype have been found in *several countries in Europe and in Asia*

EPIDEMIOLOGY —In Australia, *Rattus conatus* (*R culmorum*) is the most important carrier host, but serological evidence of *infection of bandicoots has also been found* Kmety (1957) isolated strains from hedgehogs and from *Apodemus flavicollis* in Czechoslovakia

L australis A is closely associated with infection in sugarcane fields in Queensland (Johnson, 1950, Doherty *et al*, 1956, Derrick, 1956) For instance, Doherty *et al* found that 28 out *of 42 infections in a cane-cutting region* were due to *L australis A* and one other to the closely related Esposito type (p 170), 38 out of 42 of the patients had been cutting cane

In Derrick's analysis of 219 cases of leptospirosis during

1950-54, 48 were due to *L. australis A*, and he found that 40 of these 48 worked in cane fields and the other 8 were closely associated with that work. Cases occur along the course of rivers and there are often outbreaks approximately 10 days after heavy rain. In dry weather, burning the sugar cane before it is cut is believed to be beneficial by destroying leptospires on the surface of the ground and on the plants. However, if heavy rain follows the burning there is experimental evidence that leptospires may be washed out of the soil below the surface of the ground (Smith and Self, 1955).

CLINICAL ASPECTS.—Doherty (1955) gave a full account of the clinical features of 38 cases infected by *L. australis A*. Fever and headache, not localized to any particular area, were the commonest presenting symptoms. The patients usually also complained of general muscular pain in the back and limbs, but the pain was most marked in the calves of the legs, and this was a sign of diagnostic value. Abdominal pain, sometimes very severe, was often present. Photophobia was often noted.

Respiratory symptoms were less frequent and urinary symptoms were uncommon. A slight degree of jaundice was seen in only three of the patients and two showed bile in the urine. Thirteen patients showed tenderness of the liver and in some of these the liver was palpably enlarged. Five showed splenomegaly and two had a macular rash which lasted up to 24 hours. Neck stiffness was noted in only 3 patients. Lymphadenopathy was relatively common and affected the cervical or inguinal groups of glands, or both. This is a feature which has been noted by other Australian observers, although it is not common in many other forms of leptospirosis. One patient had an epistaxis and another haematuria. In 16 patients a secondary rise of temperature was seen after the first pyrexia had ended. The death rate is low, but convalescence is protracted.

Geographical distribution of human infections

EUROPE

BAVARIA. Rimpau (1950)
CZECHOSLOVAKIA. Kmety *et al.* (1950)
SWITZERLAND. Gsell (1946 b)

ASIA

FRENCH INDO CHINA de Lajudie and Brygoo (1953)
INDONESIA Collier (1948 a)
JAPAN Kitaoka (1951)
MALAYA Fairburn and Semple (1956)

AUSTRALASIA

QUEENSLAND Lumley (1937), Johnson (1950), Doherty *et al* (1956), Derrick (1956)
SAMOA Alicata (1949) [Not fully proved]

L muenchen

(Syn strain C 90)

This is a single strain isolated from a human patient in Germany (*Wolff, 1953 b*) *Serologically it is in the same* serogroup as *L australis A*

L pomona

(Syn *L suis*, Mezzano strain, *L monyakovi*)

HISTORY —In a dairy-farming district near the township of Pomona, North Queensland cases of seven-day fever were investigated by Clayton, Derrick and Cilento (1937) and a strain of leptospires was isolated from one of the patients The strain differed antigenically from the other serotypes with which it was compared though it showed some affinity with *L autumnalis* Later, Derrick (1942) from a more extensive study of some 80 cases came to the conclusion that the Pomona strains represented a new serotype for which he proposed the name *L pomona*

Babudieri and Bianchi (1940) isolated three strains, referred to as 'Mezzano strains', from workers in the rice fields of Northern Italy and Babudieri (1941) proved them to be *L pomona* Savino and Rennella (1944) also isolated leptospires from pigs in Argentina and they considered the strains to represent a new serotype which they proposed to name *L suis* Later however the same workers (1949 a) reported *L suis* to be identical with *L pomona*

Gsell (1944, 1946 a) showed that *L pomona* was the

causative agent of 'swineherd's disease' (maladie des jeunes porchers, Schweinehuterkrankheit) which he stated had been recognized as a clinical entity in the Upper Savoy by Bouchet in 1914. The disease was well known in Switzerland as a form of serous meningitis. No bacterial cause could be found and the aetiological agent was thought to be a virus by Durand *et al* (1936 a) who used blood from patients suffering from the infection for pyrotherapy in mental disease.

EPIDEMIOLOGY.—It had long been recognized that pigs played some essential part in the dissemination of swineherd's disease, and Durand *et al* (1936 b) had transmitted the disease to young pigs. After an incubation period of 5 to 8 days the animals suffered from a short attack of fever, during which their blood was infective to man. The pigs suffered no other ill effects, except for temporary loss of appetite.

L pomona was isolated from pigs slaughtered at Brisbane, Australia, by Johnson (1939) and from pigs in Batavia by Mochtar (1940). Gsell and Rimpau (1944 a) found agglutinins for *L pomona* in the blood of 15 out of 65 pigs from St Gallen, Switzerland, where the disease is common in man, whereas no agglutinins were present in 34 pigs from Munich, Germany, where the human disease is unknown.

Gsell (1952) compared the distribution of the human disease in the different Cantons of Switzerland with the distribution of pigs and the size of herds. He found that high incidence was associated with large herds and not with the total number of pigs in a Canton. The aggregation of pigs in large herds is a modern tendency in certain areas, and its effect was shown in the four Cantons of Thurgau, St Gallen, Luzern and Waadt. Of all the human cases of pomona fever which occurred in Switzerland from 1943-49, 67 per cent were in these Cantons. Only 36 per cent of the total number of the country's pigs were in these areas, but they represented 81 per cent of all herds containing 200 pigs or more. Experimental infection of pigs is further recorded in Chapter XVI.

By contrast, in Eastern Slovakia where pomona fever is common among agricultural workers, Kmety (1957) found that *Apodemus agrarius* was the primary carrier host, and that pigs were of secondary importance except as a cause of infectio among butchers

Cattle are susceptible to infection with *L pomona* and acting as temporary carriers are responsible for human infections among workers on dairy farms In the U S A, Reinhard (1953 a) recorded bovine leptospirosis due to this serotype in 28 States Horses and sheep occasionally become infected but their possible rôle as transmitters has not been investigated Strains have also been isolated from a rat (Babudieri and Bianchi, 1940), a dog (Collier, 1948 b), *Mus musculus* (Fraga de Azevedo *et al*, 1951), *Apodemus agrarius* by Borg-Petersen and Fennestad (1956 b), *Microtus arvalis, A sylvaticus* by Popova and Amossenkova (1957) and *Paradoxurus hermaphroditus* by Gordon Smith and Broom (to be published) Serological evidence of infection was found in dogs in Australia by Oxer (quoted by Johnson, 1950) and in cattle in the Belgian Congo by van Riel and van Riel (1955)

In Switzerland the highest incidence was found by Gsell (1944) to be during the summer months when men worked barefoot cleaning out pig sties Bathing cases also naturally occur in the warmer months, but otherwise the disease shows no significant seasonal variation Males are much more frequently infected than females because they are more often exposed to risk Thus Johnson (1950) stated that only 3 out of 105 patients were female Similarly in a series of about 200 cases, Gsell (1952) noted that 96 per cent of the patients were male, and that 56 per cent were between 15 and 29 years of age Sporadic cases and epidemic outbreaks of pomona fever caused by bathing in pools or streams liable to contamination by pigs and cattle have been reported from a number of countries (Savino and Rennella, 1944, Schaeffer, 1951, Bordjoski, 1952, Blagoveshchenskaia, 1957)

Apart from infections contracted while bathing and the agricultural workers mentioned by Kmety, pomona fever is essentially a disease of persons whose occupations entail contact with pigs or cattle, either on farms or in abattoirs, meat processing establishments or retail shops In Gsell's series, 51 per cent of the patients tended or bred pigs, 18 per cent were land workers who also handled pigs and 14 per cent were pork butchers A similarly high proportion of the cases investigated in Argentina by Savino and Rennella (1944) were men who handled pigs, but in Australia and New Zealand the

highest incidence was among dairy farmers (Johnson, 1950, Faine and Kirschner, 1953) The occurrence of pomona fever among workers in cheese factories and in the rice fields of Northern Italy was recorded by Austoni (1953)

In France the infection of a pig attendant by *L pomona* was reported by Siguier and Poulet (1947), and 7 cases in piggery workers were reported from Paris and Nantes by Kolochine-Erber and Collombier (1950) and by Boquien, Kolochine-Erber, Hervouet and Duhamel (1950) Infection of a swineherd in Spain was found by Pedro Pons and Valenti (1952) In a review of leptospirosis in Yugoslavia from 1949 to 1951, Bordjoski (1952) reported 60 cases diagnosed serologically as infections by *L pomona* and of these 37 were due to swimming in a pool fouled by pigs Brede (1951) found a case in Germany in or near Cologne, and Kmety (1954) recorded 29 in Czechoslovakia In Argentina Savino and Rennella (1944) reported that *L suis*—later identified by themselves (1949 a) as *L pomona*—caused human infections Up to 1945 they recorded 34 of which 25 were attributed to bathing 7 to contact with pigs and 2 with cows Sandler (1949) found 5 pig sty workers infected in Israel

Beeson, Hankey and Cooper (1951) reported the first case in the U S A —in an abattoir worker—and Beeson and Hankey (1952) added two more Coffey Dravin and Dine (1951) found an infection in a cattle herdsman in Texas, and Schaeffer (1951) recorded that a group of young adults were infected by swimming in a stream where cattle and swine pastured In New Zealand human cases were first recognized by Kirschner *et al* (1952), and Faine and Kirschner (1953) reported that 59 cases were diagnosed the following year

Clinical Aspects —The clinical picture has been analysed by Gsell (1952) and by Frey (1948) The usual incubation period of 7 to 14 days was confirmed in the infections induced for treatment of mental patients, in whom the period was about 12 days In general, the illness showed acute onset, high biphasic temperature with meningitis, but jaundice was rare Severe headache and pains in the neck, back, joints or calves of the legs were usual Less commonly the attack began with nausea, vomiting and dizziness These symptoms, accompanied by constipation or diarrhoea, usually occurred later

Blood pressure was reduced and the pulse rate relatively slow Photophobia and conjunctivitis were common Iridocyclitis and uveitis were rare, but sometimes occurred during convalescence or after recovery Maculo-papular rashes occasionally appeared Minor degrees of hyperaesthesia or muscular paresis occurred and one instance of encephalitis was recorded An unusual case in which arthritis and myocarditis were the principal features was described by Sutliff, Shepard and Dunham (1953) Richardson (1953) recorded a case of encephalitis leading to residual weakness of one hand

The leucocytes in the blood may be reduced at first, and show moderate polymorph increase later The changes in the urine are those of slight nephritis and in the cerebrospinal fluid of moderate meningitis with *inter alia* increase of monocytic or segmented leucocytes

The illness lasts as a rule 3 to 8 days, but convalescence may take a month or more Death has occurred only very rarely, as in a man aged 73 who developed circulatory failure and bronchitis (Dotti, 1949) Subclinical infections were detected by serological tests in abattoir workers in Brisbane by Johnson (1950)

Geographical distribution of human infections

EUROPE

AUSTRIA Puntigam and Till (1950)
CZECHOSLOVAKIA Kmety (1954)
FRANCE Boquien *et al* (1950)
HUNGARY Alfoldy and Fuzi (1950)
ITALY Babudieri and Bianchi (1940)
RUMANIA Combiescu *et al* (1957)
SPAIN Pedro Pons and Valentí (1952)
SWITZERLAND Gsell and Rimpau (1944 a)
YUGOSLOVIA Bordjoski (1952)

ASIA

INDONESIA Collier (1948 b)
ISRAEL Sandler (1949)
MALAYA Fairburn and Semple (1956)

AMERICA

ARGENTINA Savino and Rennella (1944)
CHILE Kraljevic *et al* (1956)
U S.A. Beeson *et al* (1951), Schaeffer (1951)

AUSTRALASIA

AUSTRALIA Clayton *et al* (1937), Johnson (1950), Derrick (1956)
NEW ZEALAND Richardson (1953), Faine and Kirschner (1953)

CHAPTER XI

EPIDEMIOLOGY, PATHOLOGY AND CLINICAL ASPECTS OF LEPTOSPIROSIS DUE TO OTHER SEROTYPES (continued)

L grippotyphosa	*L bataviae*
L hebdomadis	*L paidjan*
L medanensis	*L semarang*
L wolffii	*L andaman A*
L hardjo	*L hyos*
L sejroe	*L celledoni*
L saxkoebing	*L mini*
Robinson type	Esposito type
Kremastos type	Valbuzzi type

L grippotyphosa

(Syn *L andaman B*, type CH 31, Duyster strain, *L bovis*, *L geffeni*, Naarandukula group)

History—In 1928 and 1929 Tarassoff (1931) cultured strains of leptospires from the blood of field workers in the Province of Moscow He showed that they differed serologically from *L icterohaemorrhagiae*, *L hebdomadis* and *L autumnalis*, and he named the serotype *L grippotyphosa* He named the disease leptospirosis grippotyphosa aquatilis and he considered it to be endemic with occasional epidemic outbreaks Tarassoff found leptospires which were morphologically similar to *L grippotyphosa* in ponds, marshy fields and wells but he did not state whether he tested them serologically Tarassoff (1934, 1935) gave good accounts of the epidemiology and clinical features of the disease which had been long known to occur during harvesting in damp fields and was called field fever or water fever

The search for human infections by *L grippotyphosa* was then pursued in Germany where mud fever (Schlammfieber)

had been known clinically as a wide-spread disease since the end of the last century It occurred along the margins of all the rivers and was recognized to be a summertime epidemic disease affecting agricultural workers Prausnitz and Lubinski (1926) had observed leptospires in a blood culture from a patient suffering from mud fever They failed to subculture the strain or to infect animals with it, so they refused to commit themselves as to its pathogenic significance

Kathe (1928, 1929) had believed that mud fever was a mild form of Weil's disease However, after Tarassoff's report, investigations by Rimpau, Schlossberger and Kathe (1938) showed that *L grippotyphosa* was the cause of mud fever in Silesia in the basin of the River Oder, of harvest fever (Erntefieber) in Lower Bavaria and Upper Palatinate in the Danube basin, and in Saxony in the basin of the Elbe Swamp fever (Sumpffieber) and water fever are yet other names for the disease Schuffner (1938) reported that the Andaman B serotype (Strain CH 31) isolated by Taylor and Goyle (1931) in the Andaman Islands was *L grippotyphosa*

Strains of leptospires isolated in Israel from diseased cattle by Bernkopf, Olitzki and Stuczynski (1947) were thought to represent a new serotype Human infections were reported by these workers, by Schachtel (1948) and by Jacusiel *et al* (1948) Btesh (1947) suggested the name *L bovis* for the serotype, but Wolff and Bohlander (1952) and van der Hoeden (1953 a) showed that it was in fact *L grippotyphosa* Other strains isolated in Israel from man and from *Microtus guentheri* by Olejnik and Shneyerson (1952) were named *L geffeni*, but these also were proved by van der Hoeden (1953 a) to be *L grippotyphosa* Alexander *et al* (1955) isolated, from *Rattus* [illegible] which [illegible] & b) demonstrated that field mice (*Microtus arvalis*) were the source from which certain children in the Netherlands became infected with *L grippotyphosa* The mice had bitten some of the children and infection probably took place because the mice voided urine on the punctured skin They found *L grippotyphosa* in other field mice and concluded that mud fever is contracted from field mice as Weil's disease is from rats Up

CHAPTER XI

EPIDEMIOLOGY, PATHOLOGY AND CLINICAL ASPECTS OF LEPTOSPIROSIS DUE TO OTHER SEROTYPES (continued)

L grippotyphosa	*L bataviae*
L hebdomadis	*L paidjan*
L medanensis	*L semarang*
L wolffii	*L andaman A*
L hardjo	*L hyos*
L sejroe	*L celledoni*
L saxkoebing	*L mini*
Robinson type	Esposito type
Kremastos type	Valbuzzi type

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The search for human infections by *L grippotyphosa* was then pursued in Germany where mud fever (Schlammfieber)

of human cases occurred, the infection rate in the field mice was between 40 and 80 per cent In non-epidemic areas it was only from 5 to 8 per cent To allow the findings to be analysed further, the mice were divided into three groups according to weight, to give an estimate of their relative ages The highest rates of infection in all districts occurred in the oldest age group At autopsy the kidneys of the infected mice were pale and swollen Microscopically there was severe interstitial nephritis with damage to the whole nephron, and leptospires could be seen in both the glomeruli and tubules The liver also was severely affected and the infection was clearly of a virulent type

Two months later a second survey was made of one particular field in which the mice had previously shown an infection rate of 80 per cent On the second occasion the mouse population was much decreased and only 27 were caught No leptospires were seen in the kidneys of any of these mice, although 20 per cent of them contained agglutinins in the blood From this work it appears that, in this district at least, the mice are subject to acute epizootic infections (for reasons that are unknown) and that they heavily contaminate the ground, water and plants Mice which recover from the epizootic may excrete the organism less intensely or for shorter times than is common in rats carrying *L icterohaemorrhagiae*

Infections by *L grippotyphosa* may be very numerous in some regions For instance, Rimpau (1948) recorded 663 cases during the years 1937-43 and 1946-47 in South Bavaria, and Popp (1950) found approximately 300 in the epidemic among pea harvesters in Lower Saxony Hermannsen (1954) reported at least 300 among pea harvesters and cabbage gatherers in Schleswig-Holstein in 1952, and Olejnik and Shneyerson (1950) recorded more than 1,000 cases in vegetable growers in Israel In 1949, leptospirosis was detected in 39 pea harvesters in Oldenburg, Holstein Glattkowski (1950) reported the outbreak, and believed it to be due to *L canicola*, although dogs were not obviously connected with the outbreak and the season was dry Fuhner (1953) however tested serum from three of the patients about a year later and came to the conclusion that the epidemic was most probably caused by *L grippotyphosa*

As well as notable epidemics smaller groups of cases are

to the end of 1942 the same authors (1943) found 21 human cases of this form of leptospirosis in the Netherlands The discovery of *L grippotyphosa* in field mice was confirmed in Germany (Kathe, 1943, Uhlenhuth, 1943 a & b) and the importance of field mice in the causation of field fever is generally accepted Kathe (1945) however believes that in Silesia the occurrence of the disease is more dependent on summer floods and standing water than on the numbers of infected mice However, Rimpau (1937) emphasized that infections sometimes occurred on ground that was not swampy, and at times other than harvest

Other rodents known to be carriers are *Apodemus sylvaticus, Clethrionomys glareolus* (Rimpau, 1950), *Cricetus cricetus* (Popp, 1950) *Microtus guentheri* (Olejnik and Shneyerson, 1950), *M agrarius, Sicista betulina* (Krassilnikov, 1957) and *M oeconomus* (Terskich, 1957)

Infection in domestic animals is considered in Chapter XVI The serotype shows a low pathogenicity for guineapigs and hamsters

The incidence is strongly seasonal and large numbers of cases are most likely to occur during harvest time Hermannsen (1954) in the epidemic in Schleswig-Holstein found a first peak of cases in August during pea harvesting and another in October and November when the cabbages in the same district were gathered

An increase in the infection rate in rodents and increase of wetness of ground and vegetation (due to flooding, rain or dew) are often major factors in the causation of epidemics in human beings The study of an epidemic among pea harvesters in Lower Saxony during July 1949 provided useful information on these points (Litzner and Hahn 1950, Popp, 1950) All the patients picked peas or handled newly cut haulms The season was warm and showery and the picking was done while the plants were still wet with dew

Three areas within the pea-harvesting region were mainly affected and the explanation was found in the varying degrees of leptospiral infection of field rodents in the district A total of 203 field mice, 2 wood mice, 1 house mouse and 22 field hamsters captured in different parts of the region were examined by Popp (1950) He found that in the area where large numbers

of human cases occurred, the infection rate in the field mice was between 40 and 80 per cent In non-epidemic areas it was only from 5 to 8 per cent To allow the findings to be analysed further, the mice were divided into three groups according to weight, to give an estimate of their relative ages The highest rates of infection in all districts occurred in the oldest age group At autopsy the kidneys of the infected mice were pale and swollen Microscopically there was severe interstitial nephritis with damage to the whole nephron, and leptospires could be seen in both the glomeruli and tubules The liver also was severely affected and the infection was clearly of a virulent type

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found, such as the 14 patients detected by Burggraf (1950) In a dry season from August to October 1949 these patients included field workers, bathers and 2 women who nursed a boy patient These 2 women seem to be among the rare examples of leptospiral infection passing directly from one person to another Sporadic cases of infection of men on service with the Armed Forces have been reported in France (Buckland and Stuart, 1945), and the U S A (Bigham 1953) Keal (1957) reported a case of a man who developed an *L grippotyphosa* infection in England, but the patient became ill a few days after reaching London by air from Malaya where he had been engaged in bridge building operations In Israel the rodent carrier is *Microtus guentheri* (Olejnik and Shneverson, 1950) and cattle are extensively infected Human infection has been caused by direct contact with cattle urine (Bernkopf *et al*, 1947) and also indirectly by drinking contaminated water (Jacusiel *et al*, 1948) Evidence of infection was obtained by agglutination tests in 4 out of 207 slaughterers by Bernkopf, Stuczynski, Gotlieb and Halevy (1948) In parts of Southern U S S R Terskich (1957) considered that cattle were apparently the reservoir hosts because he failed to find leptospires in an investigation of nearly 5,000 rodents although water fever was prevalent

Infection has been found from childhood to old age and the sexes appear to be equally susceptible

CLINICAL ASPECTS —The incubation period is usually from 9 to 16 days Later experience has confirmed Tarassoff's view (1934) that infection by *L grippotyphosa* belongs to the group of benign leptospiroses, with a low incidence of jaundice and few signs of meningitis apart from severe headache In many respects the course of disease resembles canicola fever Mild infections are more readily diagnosed when epidemics occur

Tarassoff commented on the clinical aspects of the disease, which in certain cases resembles 'grippe' or typhoid fever Abdominal forms have been noted in infection by other serotypes, but they are particularly common in the case of *L grippotyphosa* von Hoesslin (1941) found in German soldiers in France infections with severe muscular pain (suggesting appendicitis or peritonitis), diarrhoea or obstinate painful constipation Bastin, Cabanes and Lubetski (1953)

also commented on the enteric features of the illness which they reported from France A variety of rashes was frequent, the temperature commonly fell quickly after a week, and rose a day or two later for another 4 to 5 days Conjunctivitis and iridocyclitis sometimes occurred and also loss of hair The illness lasted 1 to 2 weeks but sometimes convalescence was slow and might continue for several weeks

The mortality has been very low in nearly all countries It was not considered a fatal disease by Tarassoff (1934), but Btesh (1947) reported death in 6 out of 17 human infections in Israel

Geographical distribution of human infections

EUROPE

AUSTRIA Puntigam and Till (1950)
CZECHOSLOVAKIA Kmety (1954)
DENMARK Borg Petersen (1944 a)
FINLAND Koulumies and Salminen (1953)
FRANCE Buckland and Stuart (1945)
GERMANY Rimpau *et al* (1938) Rimpau (1948), Popp (1950), Hermannsen (1954)
GREECE Klonizakis (1954)
HUNGARY Alfoldy and Fuzi (1950)
ITALY Mino (1942 b)
NETHERLANDS Schüffner and Bohlander (1942 a & b)
RUMANIA Combiescu *et al* (1957)
SPAIN Medina and Carmona (1952)
SWITZERLAND Gsell and Rimpau (1944 a)
U S S R Tarassoff (1931), Terskich (1951, 1957)

ASIA

ANDAMAN ISLANDS Taylor and Goyle (1931), Schuffner (1938)
ISRAEL Bernkopf *et al* (1947), Schachtel (1948), Jacusiel *et al* (1948)
MALAYA Fairburn and Semple (1956)

AFRICA

BELGIAN CONGO van Riel (1946, 1952 b)
EGYPT McGuire and Myers (1957)

AMERICA

CUBA Curbelo and Marquez (1949)
U S A Spain and Howard (1952), Bigham (1953)

is a natural carrier of *L hebdomadis*, and these mice abound in the regions where seven-dav fever occurs Ido *et al* found that one-third of the field mice examined had leptospires in the kidneys The disease occurs almost entirely in the autumn and affects people working in wet fields

CLINICAL ASPECTS —Gauld *et al* (1952) gave a good description of the type of illness in their cases which was of meningeal form There was sudden onset with headache, fever and neck rigidity Fever was 102°-104°F (39°-40°C) and lasted for 6 to 8 days, this was followed by a remission for 24 to 48 hours and then a recurrence for 3 to 4 days Meningitis appeared during the secondary fever Severe headache and backache were usual, and a few patients had nausea, vomiting and diarrhoea The leucocyte count was from 4,400 to 11,500 per c mm with a mean figure of 8,000, and up to 80 per cent of the cells were polymorphonuclear The cerebrospinal fluid was normal when the patients were admitted to hospital but during the secondary rise of temperature 50 to 300 cells per c mm were found—mostly lymphocytes The glucose concentration was normal and the protein content ranged towards the upper normal limits Liver-function tests were normal except in one case which showed a slight increase of bilirubin There were no deaths in this series, and no sequelae except muscular weakness which persisted in one case for three months However, as noted above, Vaucel (1938) reported one fatal case from Indo China

Geographical distribution of human infections

EUROPE

GERMANY	Mochmann, Kathe and Kuppi (1956)
U S S R	Terskich (1951)

ASIA

FRENCH INDO CHINA	Vaucel (1938)
INDONESI	[illegible] (1941)
JAPAN	
MALAYA	
NORTH I	
OKINAWA	Gauld *et al* (1952)

L medanensis

This serotype belongs to the Hebdomadis group, and was first isolated from a dog in Medan, Indonesia (Schuffner *et al*, 1935) and later found in human infections in Australia (Smith, Brown, Tonge, Sinnamon, MacDonald, Ross and Doherty, 1954, Derrick, 1956) and Malaya (Trimble, 1954) It produces a mild form of leptospirosis in man

L wolffii

This serotype also belongs to the Hebdomadis group, and was first isolated from a human being in Indonesia in 1937 (Schüffner *et al*, 1939) It has been found in human infections in Malaya (Trimble, 1954) Five Malayan strains studied by Alexander *et al* (1955) were found to represent the incomplete biotype of *L wolffii* and were designated *L wolffii* (A)

L hardjo

A single strain of the Hebdomadis group isolated from a human being in Sumatra in 1938 (Wolff, 1953 b)

L sejroe

[illegible]

on the fourth day of illness a new serotype which they named *L sejroe*, and which they found to be related serologically to *L hebdomadis* and *L medanensis* Later, Borg Petersen (1944 b) showed that all three are also related to *L saxkoebing* and to *L wolffii* These five serotypes constitute the most important members of the Hebdomadis serogroup

EPIDEMIOLOGY A strain of *L sejroe* was isolated by Borg-Petersen and Christensen from a field mouse which they considered to be *Mus musculus spicilegus* Borg Petersen (1949) recorded isolations from 16 out of 164 mice of the same species caught in various parts of Denmark However, Salminen (1956) refers to the work of Mohr (1950) who studied the

distribution of the various subspecies of *M musculus* Mohr stated that *M musculus spicilegus* is limited to South Eastern Europe, and that the subspecies found in Northern and Eastern Europe is *M musculus musculus* It would appear therefore that the natural host of *L sejroe* in Denmark is *M m musculus*

Rimpau (1943) found serological and microscopical evidence of infection with *L sejroe* in two *Apodemus sylvaticus* in Germany Evidence indicating the infection of cattle and horses is mentioned in Chapter XVI On epidemiological grounds Karakasevic (1955) thought that domestic animals might have infected a stream in Yugoslavia In this instance however the animals were not examined, nor were the small rodents in the vicinity investigated for the presence of carriers The serotype has a low degree of pathogenicity for guineapigs

In Denmark, cases occur in rural areas and are particularly associated with farming During 1943 there was a plague of mice and 198 human infections were reported in that year—almost as many as in all the other years from 1935–48 combined Borg Petersen considered that infection generally took place through injured or apparently intact skin but that sometimes it might have been due to food contaminated by mouse urine In a series of 20 cases in Switzerland analysed by Gsell (1952) there were 8 field workers, 7 cheesemakers, a pigkeeper, a butcher, a forester, a mason and a soldier An outbreak of 57 cases among children was traced to bathing in the infected stream in Yugoslavia mentioned above

Males are more commonly infected because they are more likely to be exposed There is no indication of a difference in incidence when the sexes are equally at risk, and 34 per cent of the first 285 cases reported in Denmark were women

Borg-Petersen found that the incidence in Denmark was low from January to July, then rose steeply in August reaching a maximum in October, after which it declined again The rise coincided with the harvesting of crops which takes place in August and September Harvesting is carried out predominantly by men, and the cases occurring in August are chiefly in males The female incidence begins to increase in September when the mice start migrating to the farm buildings following their food supply

CLINICAL ASPECTS —Clinically, sejroe fever resembles the

other benign forms of leptospirosis, including canicola fever In a series of 285 cases Borg-Petersen noted definite jaundice in 8 per cent, and scleral jaundice alone in a further 4 per cent A diphasic rise of temperature severe headache, muscular and neural pains were common, together with mild degrees of meningitis and nephritis On the whole, Borg-Petersen classes the disease as one of the mildest forms of leptospirosis There were however three deaths in his series one of these patients was a man aged 54 who died on the seventeenth day of illness from bronchopneumonia and another was a woman aged 47 whose death occurred on the ninth day Neither of these patients was jaundiced

Mortensen (1940) described complete flaccid paralysis of the legs which began three weeks after the onset of infection with *L sejroe*, recovery was complete after another six weeks Mortensen quoted four other cases in which there was flaccid paralysis of the legs and two of these patients also suffered temporarily from paralysis of the bladder

Geographical distribution of human infections

EUROPE

AUSTRIA Puntigam and Till (1950)
CZECHOSLOVAKIA Kmety (1955 b)
DENMARK Borg Petersen and Christensen (1939)
FINLAND Koulumies and Salminen (1953)
FRANCE Buckland and Stuart (1945)
GERMANY Rimpau (1948)
SWITZERLAND Gsell (1946 b)
YUGOSLAVIA Bordjoski (1952)

L saxkoebing

In February and March 1942, Borg-Petersen (1944 b) grew from the kidneys of two mice, *Apodemus flavicollis*, strains of leptospires which by careful serological tests he considered to be a new serotype, and which he named *L saxkoebing* after the district where the mice were caught This serotype is closely related serologically to *L sejroe* and others of the Hebdomadis serogroup

Borg-Petersen showed that three strains (two from ricefield

in Europe the disease in the rice fields and elsewhere is most commonly an acute febrile illness beginning suddenly with nausea, vomiting and abdominal pain, headache, myalgia injection of the conjunctiva and the throat also occur Relapse is rather rare, recovery almost invariable, and meningitis *infrequent Jaundice is exceptional and there is scarcely* any enlargement of the spleen, but convalescence is often prolonged by weakness (*Austoni, 1953*)

Geographical distribution of human infections

EUROPE

DENMARK Borg Petersen (1949)
ITALY Babudieri (1938), Mino (1939)
SWITZERLAND Frey (1948)

ASIA

INDONESIA Walch (1926), Walch Sorgdrager (1939), Mochtar *et al* (1941)
JAPAN Kitaoka (1951)
MALAYA Trimble (1954)
S BORNEO Lingen (1933)
THAILAND Sundharagiati and Buspavanich (1951)
VIET-NAM Kolochine-Erber *et al* (1952)

AFRICA

BELGIAN CONGO van Riel (1946)

AMERICA

PUERTO RICO and U S.A. Gochenour Yager, Wetmore, Evans Byrne, Alexander and Hightower (1951) [Not fully proved]

L paidjan

A single strain, related to *L bataviae*, isolated from a labourer in Sumatra (Wolff, 1953 a)

L semarang

A serotype first found in 1937 in a rat (*R brevicaudatus*) in Semarang Java (Sardjito and Mochtar, 1939) Single human infections in India (*Das Gupta, 1939 b*) and in Java (Collier, 1948 a) were diagnosed serologically.

L andaman A
(Syn strain CH 11)

HISTORY —A severe form of jaundice has long been known in the Indian Penal Settlement in the South Island of the Andaman Group in the Indian Ocean Chowdry (1903) gave a good account of the disease, the symptoms of which were jaundice, pains in the loins, thighs and arms congestion of the eyes, bile coloured faeces and albumin and casts in the urine At postmortem there were congestion and haemorrhages of the kidneys The patients had been exposed to rain and had been employed in cutting wood or in working on embankments or in brick fields or rice fields 588 cases occurred between 1892 and 1903, and 13 per cent of the patients died

Woolley (1911) recorded a special form of 'malaria with jaundice' from which 50 per cent of those affected died, but no malaria parasites were seen in the blood The patients were mostly convicts in 'Self Supporter' villages, and they had to stand for long periods of time in water while preparing the rice fields During 1909 Woolley saw 40 cases with intense jaundice, haemorrhagic rash, much bile in the urine and sometimes albuminuria, cerebral symptoms and conjunctival congestion Woolley (1913) stated that the disease was not malaria, and he gave clinical and postmortem descriptions which tally with those of Weil's disease, although he did not mention it by name

de Castro (1922) studied 5 cases (one of them fatal) of toxic jaundice of unknown origin He looked for *L ictero-haemorrhagiae* in the blood, but failed to find it Barker (1926) had seen some of Woolley's patients, and from 1920 to 1924 he observed other cases which had a fatality rate of 24 per cent On clinical and epidemiological grounds he considered that the illness was Weil's disease, and in three instances he saw organisms resembling leptospires in stained preparations of liver Deuskar (1928) recorded 23 cases of Weil's disease which had occurred during 1926

Taylor and Goyle (1931) made detailed investigations of the disease during an outbreak in the Andaman Islands Their work began in June 1929, and they detected 64 cases in just over four months The strains of leptospires isolated from

these cases were found to belong to two serological groups Of 28 strains 24 were identical, and were not agglutinated by the antisera prepared against a series of known serotypes Taylor and Goyle classified these strains as 'Andamans A' group, and the serotype is now known as *L andaman A* All the severe jaundiced cases (including the fatal ones) were infected by this strain

The remaining 4 strains, from nonicteric cases, resembled but were not identical with *L autumnalis* These, of which the type strain was CH 31, were known for a time as 'Andamans B,' but Schuffner (1938) found that they were identical with *L grippotyphosa*

A small number of infections by *L andaman A* has been diagnosed in Finland by Koulumies and Salminen (1953)

EPIDEMIOLOGY Leptospires were found by Taylor and Goyle in water from rice fields or swamps when the *p*H was 6 9 or over, but were not proved to be serologically the same as the strains isolated from patients Rats were examined but lepto spires were never found in their kidneys No animal carrier host has been found in the Andaman Islands, and it is supposed therefore that infection may be spread by men contaminating the wet ground by urination

The disease had a seasonal prevalence during the months of July to October which coincides with the later part of the south west monsoon, some cases however occurred in each month of the year The patients were almost all adult males who had been engaged in outdoor occupations which involved working in water and mud, as in rice fields and in the con struction of embankments The cases were scattered in the swampy areas of the Penal Settlement, but occasionally a concentrated outbreak would occur

CLINICAL ASPECTS —Taylor and Goyle observed and analysed very carefully the clinical and laboratory findings in their patients Their records agree in almost all respects with records of infection by *L icterohaemorrhagiae*, except that the Andaman strains were only slightly virulent to adult guineapigs, so that from only 1 out of 23 patients was a fatal infection transmitted to a guineapig by blood or urine The virulence of these strains was increased by animal passage There were 12 deaths among the 64 patients (20 per cent) Jaundice was present in all the

fatal cases and some of the deaths were due to cardiac failure Another interesting feature was that 8 of the 12 deaths occurred between the fifth and eleventh days of illness

Geographical distribution of human infections .

EUROPE

FINLAND Koulumies and Salminen (1953)

ASIA

ANDAMAN ISLANDS Taylor and Goyle (1931)

L hyos

(Syn *L mitis* Johnson, strain DV A, *L tarassovi*)

HISTORY —Johnson (1942) isolated in Queensland, Australia, strains of leptospires which differed serologically from all the other serotypes with which he was able to compare them and he proposed to name them *L mitis* Previously however Mino (1938) had used *L mitis* to designate strains isolated from human cases of leptospirosis among workers in the rice fields of Northern Italy These strains were shown by Gispen and Schuffner (1939 b) to be identical with *L bataviae*, and *L mitis* Mino therefore became a synonym of *L bataviae* The specific epithet *mitis* was however effectively published by Mino, in accordance with the rules of the International Bacteriological Code of Nomenclature *L mitis* Johnson is thus a homonym and cannot validly be used Savino and Rennella (1944) proposed the name *L hyos* for a serotype isolated from pigs in Argentina and Babudieri (1951 a) and Savino and Rennella (1950/53) showed that *L hyos* and *L mitis* Johnson are serologically identical Since *hyos* is thus the 'oldest legitimate epithet', it becomes the correct designation of the serotype, and *L mitis* Johnson (*non* Mino) is considered a synonym of *L hyos*

EPIDEMIOLOGY — Gordon Smith and Broom (to be published) isolated strains from *R bowersi* in Malava, but pigs appear to constitute the main reservoir hosts Johnson (1950) and Kirschner (1954) have found serological evidence of infection among cattle in Australia and New Zealand respectively, and Savino and Rennella (1949 c) reported that the sera of 5 per cent of horses in Argentina gave evidence of infection.

Human cases occur mainly among dairy farmers, pig breeders, butchers, meat inspectors, veterinary surgeons, and others whose employment brings them into contact with cattle, pigs or meat products The epidemiology of this infection is therefore essentially the same as that of pomona fever.

CLINICAL ASPECTS – The disease produced by *L hyos* runs a mild course, the outstanding features being fever, headache, conjunctivitis, muscle pains, stiffness of the neck and signs of meningeal irritation *Jaundice was absent in all the cases* observed by Gsell and Wiesmann (1948) No deaths have been reported, but Gsell and Prader (1953) described a case in which the brain was permanently damaged in a boy, three years old, who had been infected by falling into a pit containing liquid manure draining from a nearby pig sty

Geographical distribution of human infections

EUROPE

FRANCE Mattei, Kolochine Erber, Aviernos, Recordier, Payan and Barbe (1950)
ITALY Austoni (1953)
RUMANIA Combiescu *et al* (1957)
SWITZERLAND Gsell (1952)

AMERICA

ARGENTINA Savino and Rennella (1944)

AUSTRALASIA

AUSTRALIA Johnson (1942), Derrick (1956)
NEW ZEALAND Kirschner (1954)

L celledoni

This serotype was isolated from workers in sugarcane areas in Queensland, Australia (Queensland, *1953*, *Derrick, 1956*) It was established as a separate serotype by Broom and Smith (1956) who showed that it had slight affinities to *L javanica* and *L poi* Serological evidence of infection by this serotype *was noted in one case in* Malaya *by* Fairburn and Semple (1956), ... of three strains from ... ognised have been

L mini

(Syn 'Szwajizak' type ; strain Sari)

Among 89 strains of leptospires isolated in Queensland, Australia, and studied by Smith *et al* (1954), 18 belonged to the Hebdomadis serogroup They comprised two separate serotypes which were referred to as the 'Szwajizak' and 'Kremastos' types respectively, but their serological characters were not fully investigated by the Australian workers Babudieri (1956) made a systematic study of the 'Szwajizak' type and concluded that it represented the incomplete biotype of a new serotype The complete biotype was represented by strain Sari which, Babudieri stated was isolated in 1941 by Mino from a patient in North Italy Babudieri proposed to name the serotype *L mini* subdivided into *L mini* (A), the 'Szwajizak' type, and *L mini* (AB), strain Sari

The disease produced in man is mild in character, and the serotype showed a low virulence for guineapigs

ADDITIONAL SEROTYPES

Full details have not yet been published of the serological characteristics of certain groups of strains isolated from human infections in the sugarcane region of Queensland, Australia For convenience therefore they are considered together, although they are not related antigenically to one another

Robinson Type

The strains of this serotype belong to the Pyrogenes serogroup but are not identical with either *L pyrogenes* or *L australis B* (Smith *et al*, 1954, Derrick, 1956) The first strain was isolated in 1951, and a total of 14 cases had been recognized up to 1956 (Queensland, 1956) The same report noted that a positive agglutination reaction with this serotype was obtained with the serum of a *Thylacis obesulus* The illness produced is mild in character

Kremastos Type

This serotype is a member of the extended Hebdomadis serogroup, and was first isolated in 1952 (Smith *et al*, 1954)

Human cases occur mainly among dairy farmers, pig breeders, butchers, meat inspectors, veterinary surgeons, and others whose employment brings them into contact with cattle, pigs or meat products The epidemiology of this infection is therefore essentially the same as that of pomona fever

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CHAPTER XII

CLINICAL AND LABORATORY DIAGNOSIS

CLINICAL DIAGNOSIS

As in other infectious diseases, patients suffering from leptospirosis may show all or only a few of the clinical features which may be produced by the serotype involved, and only a minority of serotypes produce fatal or severe illness The likelihood of jaundice is the basis of a useful classification made by Gsell (1952) and modified in Table XXI

TABLE XXI

DEGREE OF SEVERITY OF DISEASE CAUSED BY PRINCIPAL SEROTYPES

I Most frequently icteric—most severe	*L icterohaemorrhagiae*
II Less frequently icteric—less severe	*L andaman A* *L australis A* *L australis B* *L autumnalis* *L bataviae* *L pyrogenes*
III Usually anicteric—benign leptospirosis	*L ballum* *L canicola* *L grippotyphosa* *L hebdomadis* *L hyos* *L pomona* *L sejroe*

ⁿ section a general review of diagnostic clinical features ᵗle and greater detail is given for the separate serotypes VIII, IX X and XI It is emphasized that *iorrhagiae* and other serotypes which cause jaundice many mild cases, including subclinical infections only by bacteriological and serological tests

ns are divided into three degrees according to

It causes a mild illness, and 36 cases had been diagnosed up to 1956. One strain was isolated from *Parameles nasuta*, and another from *Thylacis obesulus* (Queensland, 1956).

ESPOSITO TYPE

This serotype, of which only two strains have been isolated, was first obtained from a fairly severe anicteric case of leptospirosis in 1954. It is related to, but not identical with, *L. grippotyphosa* (Smith and Brown, 1955).

VALBUZZI TYPE

This serotype is antigenically related to *L. australis A* and the only human strain was isolated from a mild case in 1954. A serologically identical strain was isolated from the 'cane trash' on the sugarcane farm where the patient had been working before he fell ill (Smith and Brown, 1955).

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III Usually anicteric—benign leptospirosis	*L ballum*
	L canicola
	L grippotyphosa
	L hebdomadis
	L hyos
	L pomona
	L sejroe

In this section a general review of diagnostic clinical features will be made and greater detail is given for the separate serotypes in Chapters VIII, IX, X and XI It is emphasized that *L icterohaemorrhagiae* and other serotypes which cause jaundice also produce many mild cases, including subclinical infections detectable only by bacteriological and serological tests

If infections are divided into three degrees according to

severity, the clinical features in each degree might be grouped as follows

DEGREE I—most severe including almost all fatal cases

Tendency to sudden onset with fever, headache, prostration, muscular pains and tenderness, conjunctivitis, sore throat, jaundice, moderate meningitis, acute nephritis with rapidly rising concentration of blood urea

DEGREE II—less severe and rarely fatal

Onset abrupt or more insidious, with fever but less prostration, and less frequent and severe jaundice and less severe nephritis than in Degree I Meningitis is usually the central feature with headache, conjunctivitis and various forms of skin rash Severe abdominal pain may be the chief complaint Temperature often shows a remission for 24 to 48 hours at the end of the first week of the illness, the relapse may last for 3 days and the meningitis may be most severe at that time

DEGREE III—least severe and not fatal

Onset may be sudden, with fever, muscular pains sore throat, slight or no jaundice and slight or no nephritis The patient may be ambulant and the illness considered to be influenza

DIFFERENTIAL DIAGNOSIS

Until bacteriological and serological methods have fully determined the diagnosis, a differentiation from other diseases on clinical evidence will be necessary *The importance of an occupation or amusement which entails risk of contact with rats, dogs, pigs, field mice and other animals likely to harbour leptospires must always be remembered*

BEFORE JAUNDICE HAS APPEARED —In forms of leptospirosis which develop as generalized disease in contrast with the markedly meningitic, abdominal or renal forms, the following conditions will need to be considered

1 *Influenza* is not likely to produce early nephritis and meningitis, and it causes leucopenia and relative lymphocytosis rather than polymorph leucocytosis

2 *Typhoid* and *paratyphoid* fevers begin more gradually,

cause enlargement of the spleen much more often than leptospirosis and show leucopenia

3 *Rheumatic fever* causes pains in a succession of joints rather than in muscles, and there is more perspiration than in leptospirosis It is much more likely to produce endocarditis myocarditis and serositis and does not cause conjunctivitis or uveitis

4 *Brucellosis* more often causes enlargement of the spleen with lymphocytosis rather than polymorph leucocytosis

5 *Septicaemia* due to streptococci, pneumococci or other pyogenic bacteria is less likely to cause muscular pain and tenderness or conjunctivitis, and the causative organism can usually be grown by blood culture

6 *Acute nephritis* (due to haemolytic streptococci, etc) is less likely to be accompanied by muscular pains and tenderness or by conjunctivitis and uveitis

7 *Malaria* shows a characteristic fever in benign tertian and quartan forms In all forms (including malignant tertian) parasites can generally be demonstrated in the blood during or soon after attacks at any stage of the disease

8 *Relapsing fever* may be diagnosed by finding spirochaetes in the blood during the period of pyrexia

9 *Dengue* does not show signs of renal disease or albuminuria

10 *Scrub typhus* is often accompanied by an eschar at the site of infection, and the disease usually has a slower onset Vomiting and meningeal involvement are less common than in leptospirosis

11 *Q fever* causes severe headache, insomnia and fever, but there are usually no muscular pains, no arthritis, no rash, no haematuria and no involvement of meninges or eyes

12 *Appendicitis* has been mistakenly diagnosed when the muscular pain of leptospirosis was almost entirely confined to the abdominal muscles and was accompanied by vomiting

AFTER JAUNDICE HAS APPEARED —In generalized forms of the disease the following conditions will need to be considered

1 *Infective hepatitis* (including serum jaundice) does not give rise to nephritis is less often the cause of severe muscular pains and tenderness, and produces leucopenia and relative lymphocytosis

2 *Septicaemia accompanied by jaundice* and due to strepto cocci, pneumococci or other pyogenic organisms has less muscular pain and tenderness and a diagnosis is usually possible by blood culture

3 *Cholangitis cholelithiasis or carcinoma of the pancreas* cause obstructive jaundice but are not accompanied by muscular pains conjunctivitis or nephritis, and the liver function tests are more indicative of obstructive jaundice

4 *Yellow fever* usually shows a remission about the third day of illness—earlier than in leptospirosis—and black vomit is commoner, but clinical differentiation may be very difficult (We are reminded of this by the mistake which Noguchi was led into when he isolated a strain of leptospires from an illness considered by clinicians to be yellow fever but which most likely was Weil s disease)

5 *Malaria or relapsing fever* with jaundice can usually be diagnosed by examination of blood films

ANICTERIC FORMS —In cases with *meningitis* and rarely *encephalitis* as the main features the following conditions will need to be considered

1 *Pyogenic bacteria* and *Cryptococcus neoformans* produce meningitis (either primary or secondary) but it is rarely accompanied by conjunctivitis or skin rash The increase of cells in the cerebrospinal fluid is polymorphonuclear in most bacterial infections and lymphocytic in cryptococcal infections and the infecting organism can usually be seen or cultured. Meningitis secondary to brain abscess may be accompanied by a history of infection in an ear or elsewhere in the body The pulse rate may be slow and there may be localizing nervous signs or evidence of a space occupying lesion Meningitis secondary to infection of cranial sinuses will usually be detectable by examination of the sinuses by clinical or radio-graphical methods

2 *Tuberculous meningitis* usually begins more insidiously than leptospirosis and there is frequently a history or evidence of tuberculosis elsewhere in the body Tubercle bacilli are often found in the cerebrospinal fluid even in the first specimen examined and the concentration of glucose in the fluid is almost always reduced by the end of the first week

3 *Toxoplasmosis* causes meningitis in children by intrauterine

infection, and tends to produce internal hydrocephalus and cerebral calcification Convulsions, palsies, choroidoretinitis and rarely enlargement of liver and spleen occur A cytoplasm modifying antibody (dye test), a complement-fixation test and a skin test are available to help diagnosis

4 *Benign lymphocytic meningitis* may be due to infection by various known viruses [Most of the information in this paragraph is derived from a paper by MacCallum, 1951] In practically all known virus infections the meninges may be affected In most instances this can be detected only by finding an increase in cells and protein in the cerebrospinal fluid, but in some cases clinical evidence of meningitis is obvious When it accompanies or immediately succeeds a recognized infection such as mumps or hepatitis the diagnosis is clear When it occurs a month or more later, or is completely unrelated to such an illness, laboratory tests may be of value Viruses in this group for which tests are available include

(*a*) Lymphocytic choriomeningitis
(*b*) Mumps
(*c*) Herpes simplex
(*d*) Lymphogranuloma-psittacosis group
(*e*) Louping ill
(*f*) Coxsackie group and ECHO viruses

In most of these forms of lymphocytic meningitis there is much less likelihood of conjunctivitis, skin rash, nephritis or jaundice than in leptospirosis The diagnosis of each of the virus infections may be confirmed by serological methods and in some by animal inoculation or tissue culture MacCallum stated that in England the viruses mentioned above, toxoplasms and leptospires account for at most about 20 per cent of lymphocytic meningitis which is not tuberculous The remainder is cause[illegible] y poliomyelitis

5 [illegible] does not show conjunct[illegible] tis Ramsay (1955) pointed out, in recording four examples of meningitis due to *L. canicola*, that in leptospirosis the pleocytosis in the cerebrospinal fluid shows a rough parallelism with the protein level In poliomyelitis, increase of protein is often delayed until the second or later weeks when the cells are decreasing or have

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5 *Poliomyelitis* in a non-paralytic form does not show conjunctivitis, skin rash, jaundice or nephritis Ramsay (1955) pointed out, in recording four examples of meningitis due to *L canicola*, that in leptospirosis the pleocytosis in the cerebrospinal fluid shows a rough parallelism with the protein level In poliomyelitis, increase of protein is often delayed until the second or later weeks when the cells are decreasing or have

returned to normal. Frey (1948) gave a table of differences between benign leptospirosis and poliomyelitis, of which Table XXII is a modified form. The diagnosis of non-paralytic poliomyelitis is often inaccurate. MacCallum (personal communication) told us that in a small series of cases diagnosed

TABLE XXII

DIFFERENTIAL DIAGNOSIS OF BENIGN LEPTOSPIROSIS AND POLIOMYELITIS

(after Frey, 1948)

	Benign Leptospirosis	*Poliomyelitis*
History	Often occupational risk of infection	Occasional history of contact with other cases
Leucocytes in blood	Leucocytosis or unaltered	Usually unaltered
E S R	At first, always raised	Usually normal
Urine	At first, albumin, erythrocytes, leucocytes, casts	Normal
Lumbar Puncture (early)	At first, usually normal, sometimes raised pressure	In meningeal stage pleocytosis, at first polymorph
Lumbar Puncture (late)	*Pleocytosis* (*predominantly lymphocytic*), increase of protein roughly parallel with pleocytosis	*Pleocytosis* (*mostly* lymphocytes), increase of protein often later than first pleocytosis
Pulse	Relative bradycardia	Usually tachycardia
Blood Pressure	Usually low	Normal
Motor Power	Seldom impaired	Often impaired
Reflexes	Normal or increased, seldom weakened	Usually absent, sometimes increased
Muscle Pain	Usually prominent	Less prominent

clinically, laboratory tests showed that most of the diagnoses were incorrect. Laboratory tests for diagnosis of poliomyelitis by animal inoculation, tissue culture or serological methods are still too specialized for use in most hospital laboratories

6. *Rubella* produces enlargement of lymph glands and a distinctive rash

7 *Erythema multiforme* shows a rash which in shape and distribution is usually different from the rash in leptospirosis and includes the buccal mucosa, which is not the case in leptospirosis

8 *Stevens-Johnson syndrome* comprises a skin eruption resembling erythema multiforme, with lesions of the mouth, conjunctivae, genitalia and anus

9 *Secondary syphilis* produces a longer-lasting rash and a positive Wassermann test [The criteria recorded in paragraphs 6 to 9 inclusive are taken from Laurent *et al*, 1948]

10 *Glandular fever* with meningitis usually exhibits some other features of the disease such as enlarged lymph glands, and does not show conjunctivitis or a rash There usually is an increase of mononuclear cells (of which some have special characteristics) and a positive sheep cell agglutination (Paul-Bunnell) reaction in the blood

11 *Encephalitis due to leptospirosis may be accompanied by* some degree of jaundice, nephritis, skin rash or conjunctivitis, which helps to distinguish it from encephalitis lethargica or encephalitis secondary to vaccination, measles, mumps, or other febrile conditions

Forms with Predominantly Abdominal Features —Such forms have been found in infection with various serotypes, but especially with *L grippotyphosa* Whether abdominal pain, diarrhoea or constipation is the most obvious symptom, slight jaundice, nephritis, conjunctivitis, skin rash or severe headache will often be detectable We know of a schoolboy in whom severe epistaxis occurred after a skin incision had been made for appendicectomy and the operation was discontinued, Weil's disease was established by serological examination

Forms with Predominantly Renal Signs —Faint jaundice, conjunctival congestion skin rash, muscular pains or abnormality of the cerebrospinal fluid help to combine with nephritis to suggest leptospirosis

Forms with Fever, Malaise and Indistinct Leading Signs or Symptoms —These may be suspected to be leptospirosis if there is any occupation or amusement involving contact with rodents which are known to harbour leptospires in the country concerned

LABORATORY DIAGNOSIS

In addition to the conclusive bacteriological and serological examinations described below, certain findings are important in confirming a clinical diagnosis of leptospiral disease These are a leucocyte count, which shows an increase in polymorphonuclear cells if the count is above the normal figure, and evidence of nephritis with an increase of urea in the blood During the first few days of a leptospiral infection, leucopenia has been observed, but patients are not usually seen so early in the course of their illness When the leucocyte count is altered in leptospirosis (as it almost always is in severe and *moderately severe cases)* a *polymorph leucocytosis* is the usual finding This contrasts with the reduction of leucocytes and relative increase of lymphocytes and monocytes in infective hepatitis, as described for example by Findlay, Dunlop and Brown (1931) In our experience there have been total leucocyte counts rising to 33,800 per c mm with 90 per cent neutrophil polymorphs It should be remembered however that in some stages of the less severe forms of leptospirosis, including meningeal forms, the total and differential leucocyte counts may be within normal limits In addition, the erythrocyte sedimentation test, and the concentration of glucose in the cerebrospinal fluid have some diagnostic importance (p 96 & 100)

Laboratory diagnosis depends principally on demonstrating *the infecting leptospires in tissues or fluids from* the patient, or on detecting in his serum antibodies which react specifically with a known serotype *

Demonstration of Leptospires

1 By examination of blood or other fluid or ground tissue by dark ground microscopy, or of stained films of such material

2 By culture of blood or other fluid, or of tissue taken post-mortem

3 By animal inoculation, and subsequent culture from the infected animal

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* J W Wolff has *embodied the results of his long experience in* an excellent monograph The Laboratory Diagnosis of Leptospirosis' (1954)

4 By microscopical examination of tissues suitably stained

Culture from the patient or from an infected animal is the most satisfactory of these methods, because the culture can be accurately identified serologically

1 MICROSCOPICAL EXAMINATION OF BODY FLUIDS AND GROUND TISSUE —Leptospires may be detected microscopically in the (*a*) blood, (*b*) urine or (*c*) cerebrospinal fluid of the living patient or (*d*) in ground tissues after death

(*a*) *Blood* —Leptospires are present in the blood during the first week of illness but their numbers are relatively small in human infection, and 'blood threads' ('pseudo-spirochaetes') are easily mistaken for them unless the details of morphology and motility are carefully observed If a leptospire is kept in focus for a few minutes in the dark-ground microscope, the strong light decreases its rotatory motion and the fine even coils can be more easily seen The organism can then be clearly distinguished from blood threads which are not coiled, which may bend at any part of their length and which sometimes end in a small knob but not a wide curve, the threads do not rotate round the long axis

Ruys (1933) has described a method for concentrating the leptospires in blood (Appendix) Wolff (1954) reported that by this concentration leptospires were seen in the blood of 32 out of 100 patients examined during the first eight days of illness, in contrast to 8 without concentration *L icterohaemorrhagiae* has been seen microscopically in the blood, and isolated from it as early as the first day of illness (Schuffner, 1934), and it is supposed that proliferation in the blood stream is the usual preliminary in all forms of leptospirosis Korthof (1932) injected *L grippotyphosa* into eleven human volunteers and recovered the strain from the blood of some during incubation (once as early as four days before illness developed) and up to the second day of disease A similar leptospiraemia occurs in experimental animals

(*b*) *Urine* —Leptospires are more frequently seen in the urine in severe forms of leptospirosis with nephritis They are usually present from the second week onwards, although they were found by Inada *et al* (1916) in 17 per cent of patients by the tenth day and even earlier Alston (1948) failed to demonstrate leptospires either microscopically or by guineapig inoculation in

the urine of 12 out of 13 patients tested during the first ten days of illness Leptospires were present in five cases examined respectively 6, 10, 13, 16 and 23 days after onset, no leptospires were found in five cases tested from the seventeenth to fortieth days Wolff (1954) quoted Schuffner's belief that if earlier examinations were negative, the finding of leptospires in the urine was not to be expected after the twenty first day of the disease

Records of prolonged urinary excretion of *L icterohaemorrhagiae* are rare, but include the detection of leptospiruria at the thirty-third week of illness in a very unusually prolonged infection (Murgatroyd, 1937) An instance of leptospiruria lasting for 11 months following infection by *L australis B* was noted by Johnson (1950)

Thus leptospires are found in the blood during the first week and become progressively less common later, while in the urine they are unusual in the first and second weeks and subsequently become more abundant Since the organisms may be lysed by acid urine as well as by antibodies in the urine, the patient should (if necessary and clinically permissible) be given enough alkali to make the urine alkaline, and the examination by microscopy, culture or animal inoculation should be made with the least possible delay The urine should be centrifuged for 10 minutes at 3,000 r p m after the addition of saponin in the proportion of 0 1 g per 100 ml In addition to examination by dark ground microscopy, films stained by Giemsa's stain or silver impregnation may be examined, but stained organisms are not so easily recognized as motile, live ones seen by the dark-ground microscope

(*c*) *Cerebrospinal Fluid*—Leptospires may occur in the cerebrospinal fluid towards the end of the first week of illness but are usually few and not readily found by microscopical examination

(*d*) *Ground Tissues*—Suprarenal gland, kidney and liver are the best tissues for dark-ground preparations or stained films

2 Culture of Body Fluids and Tissues—Cultures can be made from the various body fluids at the periods of the disease noted above for microscopical examination, and several blood specimens should be cultured during the febrile period The development of suitable media for culture is outlined in

Chapter III and the preparation of media, including Fletcher's, Korthof's, Vervoort's and Stuart's is given in detail in the Appendix We have used all of these media successfully, and mention Stuart's medium since it does not require peptone which may not always be obtainable in the necessary quality Medium is usually stored in amounts of 5 to 7 ml, in tubes or bottles less than half filled The inoculum may consist of five to ten drops of patient's blood, equal amounts of serum and broken blood clot, urinary deposit after centrifuging, cerebrospinal fluid, or tissue ground in broth or in leptospiral medium Leptospires do not usually thrive in the presence of other bacteria, so if contamination is known or suspected animal inoculation is preferable

For primary culture, incubation at 37°C is recommended and cultures should be examined by dark-ground microscopy at intervals of 3 to 7 days When growth has been detected, further incubation should be at 28°–32°C Primary cultures often develop slowly but if no growth has appeared after 4 weeks' incubation, it is unlikely to occur later The methods used to identify the strains are described in the Appendix

in guineapigs, but a large number of serotypes produce a non fatal infection with leptospiraemia from which the strains can be isolated by blood culture Syrian golden hamsters (*Cricetus auratus*) can be used for the same purpose, and unlike guineapigs they are highly susceptible to *L canicola* (Morton, 1942, Larson, 1944) Larson (1941 a) found that a Swiss strain of albino mice could be infected with *L icterohaemorrhagiae*, and Packchanian (1943) showed that the albino American Deer Mouse (*Peromyscus maniculatus gambeli*) was also susceptible Neghme, Christen, Jarpa and Agosin (1951) confirmed Packchanian's observation, but they found certain pure breeds of white mice to be resistant while others were highly susceptible More recently van der Hoeden (1954) recommended *Meriones crassus sacramenti* (a rodent common in Israel) as particularly suitable for leptospiral investigations

Inoculation of young guineapigs or young hamsters is usually made by intraperitoneal injection, or by rubbing material into the shaved and scarified skin of the abdomen, or by both

methods When water, as from swimming baths, is being examined for the presence of leptospires, guineapigs may be shaved and scarified on the abdominal wall and placed for an hour in troughs containing enough water to reach the abdomen (Appelman, 1934)

If intraperitoneal inoculation has been made, abdominal paracentesis on the fourth to sixth day *after injection may show* active leptospires in sufficient numbers for identification by dark-ground examination, and then the heart blood may be cultured to isolate the strain If leptospires have not been seen in the peritoneal fluid by the end of seven days, it is worth while culturing the heart blood at that time, or testing the animal's blood for agglutinins one month after inoculation In this way infections due to serotypes with only a low pathogenicity for guineapigs may be detected If infection of the guineapig with a pathogenic strain is allowed to follow its natural course, there is usually an increase of temperature above the normal of [illegible] from the fifth day after injection No other sign [illegible] the coat, decrease of act[illegible] (seen best on the ears an[illegible] eighth to tenth days, 12 to 48 hours before death

At postmortem examination, haemorrhages may be found in any tissue, and jaundice in almost all except the nervous system with congestion of the glandular organs The appearance of the lungs is usually very characteristic There are deep red haemorrhagic areas, irregular *in size and shape, but defined* from the surrounding lung tissue They give the organ a distinctive, spotted appearance which Inada *et al* (1916) aptly compared to the mottled wings of a butterfly Otherwise, the postmortem findings in the guineapig are very similar to those in fatal human infections

Wylie (1946 *a*) *made biochemical and histological examinations* of more than 100 guineapigs infected with a highly virulent strain of *L icterohaemorrhagiae* He found that the average value of blood urea in 20 uninfected guineapigs was 16 mg per 100 ml and the average value for 20 guineapigs eight days after inoculation with *L icterohaemorrhagiae* was 231 mg He stated that liver necrosis in infected guineapigs was a variable feature, and that a statistically significant

reduction in its incidence could be affected by administration of methionine over and above the amount contained in an adequate diet Irrespective of the liver necrosis, kidney damage seemed to be uniformly severe, and death appeared to be due invariably to renal failure The renal lesion comprised severe engorgement of the glomerulus, with tubular damage similar to that seen in various human diseases of differing aetiology such as incompatible blood transfusion, traumatic anuria, and blackwater fever Faine (1957 a) who used a highly virulent strain of *L icterohaemorrhagiae* was able to demonstrate nitrogen retention twenty-four hours after infection and he found that the blood urea level rose terminally to over 200 mg per 100 ml

In the guineapig, leptospires are numerous in the blood at the time of death whereas in human beings the period of leptospiraemia ends several days before the greatest risk of death occurs A series of experiments was made by Faine (1957 b) to determine the distribution of leptospires in the tissues When guineapigs were killed *in extremis* he estimated that 50 to 70 per cent of the leptospires were present in the blood Of those in the tissues, the liver contained about 80 per cent, the adrenal 6 per cent and the kidney 5 per cent

Sometimes the blood from a patient with Weil's disease fails to kill a guineapig or to cause severe infection or detectable leptospires, but the subinoculation of this animal's blood or tissues into another guineapig causes a typical fatal infection

The pathological changes produced in Syrian hamsters have generally been studied after infection with *L canicola*, but Brunner (1948) stated that *L icterohaemorrhagiae* gave rise to essentially the same picture Uhlenhuth and Schoenherr (1951) used a highly virulent strain of *L canicola* which caused death between 75 and 85 hours after intraperitoneal injection They found that haematuria occurred after 40 to 48 hours, by which time leptospires were present in the urine As a rule the animal showed no outward signs of illness until a few hours before death Then tonic and clonic spasms might develop, or the hamster sit motionless making only a few ataxic movements when touched Jaundice was never marked, and was often completely absent

At postmortem examination widespread haemorrhages were found in the muscles and under the serous coat of the abdominal

viscera The lungs were haemorrhagic and tended to be consolidated rather than to show the butterfly wing appearance seen in infected guineapigs In other respects the pathological findings were essentially the same in both animals

Chicks 1 to 2 days old may be used for isolating leptospires by inoculating the birds intraperitoneally with urine or other fluid and examining the heart blood by dark ground microscopy 6 days later Embryonated eggs may be inoculated for isolation of leptospires if no secondary organisms are present Allanto c fluid should be examined when the embryo is sluggish

4 Microscopical Examination of Stained Tissues — Microscopical examination of human or animal tissues stained by the Levaditi or Dobell methods will reveal leptospires in the kidneys liver suprarenal glands or other infected tissues. In sections even more than in films of urine or other body fluids the fine spirals of the leptospires are almost always filled by the stain so that identification of the organisms depends on recognizing by experience the various forms they may assume (Fig 33) In some sections the leptospires are very numerous between liver cells or in the lumen of kidney tubules or between the tubules In addition Sheldon (1945) and Gardner and Wylie (1946) suggested that muscle biopsy to detect the specific lesion of muscle fibres (p 94) may be a useful diagnostic method

Demonstration of Antibodies

1 By agglutination test with suspensions of killed leptospires of known serotype

2 By agglutination lysis test with living leptospires

3 By complement fixation test

4 By erythrocyte sensitization test

5 By adhesion test

The patient s serum may show antibodies by any of these methods in significant concentration from about the seventh day of illness in most forms of leptospirosis but in certain instances the production of antibodies is greatly delayed Thus Broom (1951 a) referred to a few cases of Weil s disease in which the agglutination reaction was negative on the twenty first day of disease but became positive at a later stage of the illness

The number of antigens that should be tested with any



Fig. [illegible]

Guinea-pig kidney showing variation in appearance of individual leptospires stained by Levaditi's method (× 700)

viscera The lungs were haemorrhagic, and tended to be consolidated rather than to show the butterfly-wing appearance seen in infected guineapigs In other respects the pathological findings were essentially the same in both animals

Chicks, 1 to 2 days old, may be used for isolating leptospires by inoculating the birds intraperitoneally with urine or other fluid and examining the heart blood by dark-ground microscopy 6 days later Embryonated eggs may be inoculated for isolation of leptospires if no secondary organisms are present Allantoic fluid should be examined when the embryo is sluggish

4 MICROSCOPICAL EXAMINATION OF STAINED TISSUES — Microscopical examination of human or animal tissues stained by the Levaditi or Dobell methods will reveal leptospires in the kidneys, liver, suprarenal glands or other infected tissues In sections even more than in films of urine or other body fluids, the fine spirals of the leptospires are almost always filled by the stain so that identification of the organisms depends on recognizing by experience the various forms they may assume (Fig 33) In some sections the leptospires are very numerous between liver cells or in the lumen of kidney tubules or between the tubules In addition, Sheldon (1945) and Gardner and Wylie (1946) suggested that muscle biopsy to detect the specific lesion of muscle fibres (p 94) may be a useful diagnostic method

DEMONSTRATION OF ANTIBODIES

1 By agglutination test with suspensions of killed leptospires of known serotype
2 By agglutination lysis test with living leptospires
3 By complement fixation test
4 By erythrocyte sensitization test
5 By adhesion test

The patient's serum may show antibodies by any of these methods in significant concentration from about the seventh day of illness in most forms of leptospirosis, but in certain instances the production of antibodies is greatly delayed Thus, Broom (1951 a) referred to a few cases of Weil s disease in which the agglutination reaction was negative on the twenty-first day of disease but became positive at a later stage of the illness

The number of antigens that should be tested with any

particular serum obviously depends on the serotypes known to be present in the locality, or on clinical indications In order to test reactions to a large number of antigens when the serotypes in a region are being explored, one of us has used mixtures of antigens which is described in the Appendix

1 AGGLUTINATION TEST —The agglutination test with killed organisms is a convenient form of reaction for routine diagnostic work, but many observers consider that living cultures should be used in all critical experimental investigations Killed suspensions suffer also from the disadvantage that their stability and sensitivity cannot be guaranteed for any fixed period, and careful controls of the suspensions must be made Sometimes a culture of a strain of leptospires will supply a satisfactory suspension for a long time, and then suddenly lose its agglutinability or become clumped in culture saline, or a serum known to be negative Broom and Brown (1943) obtained false positive reactions (up to 1/300) with blood from 34 patients who had jaundice, but not Weil's disease, when they used a particular antigenic suspension which had previously been specific Suspensions from later subcultures of the same strain were again specific

Cultures showing a sufficiently heavy growth are killed by adding formalin neutralized by pyridine or magnesium carbonate Many workers follow Schuffner's method of setting up the test in the depressions of a porcelain palette using a drop technique and making the range of final dilutions of the patient's serum 1/10, 1/30 and so on to 1/30,000 (Appendix) With positive and negative controls the mixtures are left overnight at room temperature or 5°C The reading is made by placing a drop of each dilution on a glass slide, and examining them without cover-slips by dark-ground microscopy at a magnification of ×120 using a condenser which does not require oil between it and the slide

If the test serum has a high antibody content, agglutination may be incomplete in low dilutions (prozone effect) In higher dilutions clumping is complete and the background is quite clear, but a proportion of free leptospires appear again as the end point of the reaction is approached (Fig 34) It is our custom to record the titre of the serum as the dilution in which *approximately one half of the leptospires are agglutinated* and

1 2 3 4 5

Fig 34

Microscopical appearances seen in agglutination and agglutination lysis tests

1 Contr[illegible]

[illegible] sitive reaction

Adapted by kind permission from an illustration in 'Leptospirosen' by Dr O Gsell, published by Hans Huber, Bern

about 30 per cent of the organisms are either agglutinated or lysed

(3) COMPLEMENT-FIXATION TEST —As the result of the combined experience of many workers the techniques of the agglutination and agglutination lysis tests have become standardized to a considerable extent The position with regard to complement fixation tests in leptospirosis is however quite the reverse, and even a wide variety of antigens has been advocated at different times Thus Bessemans and Nelis (1928) claimed good results when they used living cultures or aqueous or alcoholic extracts of the livers of infected guineapigs Pot and Dornickx (1936) used leptospires sedimented from culture by centrifugation They considered that the method gave results at least as good as those of the agglutination lysis test

Gaehtgens (1950) developed the test further and claimed that it could be used to distinguish serotypes as accurately as agglutination On the other hand Papageorgiu (1938) reported that a similar type of antigen reacted with antisera prepared against a number of different serotypes Yager, Gochenour, Warner, Wetmore and Hall (1951) also obtained group reactions with antigens consisting of leptospires disintegrated by sonic vibration Schneider (1955) treated washed leptospiral bodies of *L icterohaemorrhagiae* with sodium deoxycholate, and obtained a water- and ethanol soluble material which fixed complement in the presence of a wide range of leptospiral antisera Similar results were obtained by Muraschi Clemons and Tompkins (1956) with ethylene glycol extracts Schubert, Carrington, Conner and Holdeman (1956) found that washed suspensions of leptospires provided satisfactory antigens and that they were mainly serotype specific Terzin (1956) also obtained specific complement fixation reactions with boiled whole cultures, and found that the antigen was soluble in 50 per cent acetone Fuhner and Mumme (1955) noted that complement fixing antibodies disappear from the blood within a few months It has been suggested that positive complement-fixation reactions carried out on herds of cattle might indicate more definitely than agglutination tests the presence of active infection

The use of complement fixation tests has been recommended by a number of workers including York (1952) and Schubert

the other half free This is an entirely arbitrary limit, but it was chosen because it can be estimated easily

Methods have been described for carrying out the agglutination test more rapidly and for reading it with the naked eye or a hand lens We have found the ' rocker method ' described by Brown (1939) to be dependable and to give end results—as Brown stated—at one dilution lower than the method described above Bryan (1957) advocated an essentially similar method, but colours the antigenic suspension with Giemsa's stain Pot (1936) also described a rapid macroscopic agglutination test carried out in tubes All these methods need specially dense antigenic suspensions A macroscopic agglutination test with living cultures was used by Smith and Tulloch (1937) who incubated the mixtures for 3 hours at 37°C followed by 30 mins at 55°C

(2) AGGLUTINATION LYSIS TEST —In the agglutination-lysis test the dilutions of serum prepared as for the agglutination test are mixed with equal volumes of a 7 to 10 day culture of living organisms The mixtures are incubated for 2 to 4 hours at 32°C , and the readings are made by dark-field microscopy as in the agglutination test Agglutination occurs in the lower serum dilutions, but it is gradually replaced by lysis of the organisms as the dilution increases (Fig 34) In still higher dilutions the degree of lysis progressively diminishes until finally no difference in number of leptospires can be distinguished between the serum-antigen mixtures and controls without serum

Complement plays no part in the lytic reaction, which occurs equally with unheated sera and with those inactivated at 56°C As a rule, antisera react to a higher titre in this test than when formolized antigens are used

As an aid in deciding the end point of the reaction Borg-Petersen and Fagraeus (1949) recommend the inclusion of two saline controls One consists of equal parts of saline and culture (' negative control ', corresponding to the concentration of leptospires in the serum-antigen mixtures) and a second of half that density (' 50 per cent control ') The serum titre is taken as the highest dilution in which the number of free leptospires is nearer the density of the ' 50 per cent control ' than that of the ' negative control,' i e the dilution in which

INTERPRETATION OF RESULTS OF SEROLOGICAL TESTS

NEGATIVE REACTIONS —Multiple negative tests throughout the course of illness and during convalescence will exclude leptospirosis with considerable certainty, because it must be very rare to find cases such as that reported by Garnier and Reilly (1917), in which, although leptospires were isolated from the patient, no antibodies appeared in the blood Wolff (1952) pointed out that this case occurred in the early days of leptospiral research, and that no agglutination tests were performed Garnier and Reilly carried out a protection test in guineapigs, and concluded that no immune bodies were present on the twenty-ninth day of disease Wolff considered that this finding did not necessarily prove that agglutinins were also absent More recently on epidemiological and clinical evidence, Herbert Burns and Flavell (1951) and Marcuse and Pohlmann (1952) have again suggested that antibodies may be absent in cases of leptospirosis In view of the well-recognized difficulties in the clinical diagnosis of the disease, we would hesitate to accept this thesis until it has been proved by the isolation from such cases of strains of leptospires

POSITIVE REACTIONS —Positive agglutination reactions may be the result of (1) the administration of immune serum, (2) present disease, (3) the presence of residual antibodies from past infection or active immunization

(1) If therapeutic antiserum has been given to a patient before a specimen of blood is taken for examination, the fact should be reported to the laboratory The effect of such administration is however likely to be only transient because Alston (1940) found that the agglutination reaction in rabbits became negative within three days after the injection of 10 ml of antileptospiral serum per litre of plasma We tested blood specimens from the human case of influenza, described by Robertson (1946), who received approximately 40 ml of immune horse serum per litre of plasma The agglutination titre of the horse serum was 1/30,000, which would give a calculated titre of about 1/1,000 in the general circulation Samples of blood taken 5 and 60 minutes after administration showed a titre of 1/300 The level fell to 1/100 after 12 hours, and to 1/30 after 24 hours

and Martin (1956) However, in a Report of the World Health Organization (1956) the opinion was expressed that·

At the present stage of development complexities of preparation and standardization of antigens and the exacting techniques required in the performance of leptospiral complement fixation examinations limit their utilization to research laboratories familiar with the particular complement-fixing antigens they employ

(4) ERYTHROCYTE-SENSITIZATION TEST —Chang and McComb (1954) sensitized human red blood corpuscles by adding to them an ethanol fraction of leptospiral antigen, and found that the corpuscles were agglutinated when serum containing leptospiral antibodies was added The fractions obtained from *L icterohaemorrhagiae*, *L canicola*, *L pomona*, *L hebdomadis* and *L autumnalis* were genus specific since corpuscles sensitized by any of them were agglutinated by antiserum prepared against any of these serotypes Cox (1955) showed that sheep erythrocytes sensitized with the same fraction were haemolysed by antileptospiral serum in the presence of complement The reaction was genus specific and Cox suggested that the reaction might provide a useful screening test A large number of sera from known human cases have been submitted to both these tests (Queensland, 1956) The conclusion was reached that the agglutination test was not sufficiently sensitive and that the lysis test was too sensitive giving a number of apparently nonspecific reactions

(5) ADHESION TEST —This test, which was described by Brown and Davis (1927), is now only of historical interest, because it was superseded by the agglutination test In carrying out the modified test used by Brown (1935), mixtures are made containing equal volumes of (1) serial dilutions of patient's serum, (2) actively growing cultures of leptospires, (3) a 1/5 dilution of fresh guineapig serum (for complement), and (4) a thin suspension of bacteria in saline After incubation for 30 minutes at 37°C a drop from each mixture is examined by dark ground microscopy In a positive reaction, bacteria are seen firmly adherent to the leptospires, whereas with negative sera the bacteria are scattered uniformly in the field

In a series of parallel tests Brown (1935) found that patients' sera gave similar antibody titres in adhesion and agglutination tests

TABLE XXIII

RELATION BETWEEN DAY OF DISEASE AND APPEARANCE AND DEVELOPMENT OF ANTIBODIES

(Bloom 1948)

Day of disease									
2	3	4	5	6	7	8	9–13	14–20	> 20
0							300		
	0					300			
	0						1 000		
		0				100		300	
		30						10 000	
			0					300	1 000
			0					300	3 000
			0	0	10		1 000	3 000	
			0				1 000	3 000	
			30				1 000		
			100				3 000		
			300					3 000	
				0					1 000
				0					3 000
				0					3 000
				0			300		10 000
				10			1 000		
				30			100		
				30			3 000		
				30				3 000	
				30			1 000		30 000
					0			1 000	
					0			*1 000*	
					10			300	3 000
					30				10 000
					100		300	1 000	3 000
					100			3 000	3 000
					300			3 000	3 000
					1 000			3 000	
						0		3 000	30 000
						30	1 000		
						30		3 000	
						100		1 000	
						100		3 000	
						100			10 000
						300		1 000	
						300	1 000	3 000	
				0			0	100	300
			0				0		30 000

0—No agglutination at dilution of 1/10
10 30 etc Reciprocals of agglutination titres

(2) In patients who are not likely on account of their occupation or otherwise to have been infected previously, an agglutination titre of 1/300 may be regarded as diagnostic A low agglutination titre and doubt as to the exact date of onset of infection are indications for repetition of the test after a few days Recent infections show a steep rise of titre, which may reach 1/10,000 or sometimes 1/30,000 by the third or fourth week of the illness Broom (1948) analysed the results of agglutination tests made at different times after the onset of the illness in 39 patients and the results are shown in Table XXIII

These results in Weil's disease show that, as is the general experience, significant antibody levels are rarely found before the sixth day of illness and are rarely delayed beyond the twelfth day There is no apparent correlation between the time of first appearance of antibodies and the final titre Broom tested serum from 169 patients during the third or later week of illness and found the highest titre to range from 1/300 in 7 per cent to 1/30,000 in 4 per cent He did not find higher titres such as that of 1/2,000,000 reported by Senthille, de Bayo and Kolochine-Erber (1946)

The interpretation of a low titre is difficult if the findings of only one test are available As is shown in Table XXIII, titres as low as 1/300 or even 1/100 may occur quite late in the disease *In two of these patients the diagnosis was proved* correct by isolating leptospires from the urine Low readings cannot therefore be automatically regarded as resulting from past infection The correct decision can be reached only by repeated examinations Early in the disease, say up to the fourteenth day, we would consider positive agglutination even to the lowest titres to be suspicious, and recommend the testing of further specimens

(3) After recovery the titre falls at a variable rate, but may be found at 1/100 or 1/300 for many years Thus Kisker (1935) *found positive agglutinations up to 16 years after recovery*, Uhlenhuth and Fromme (1930) after 22 years, and Stuart (1939 a) after 28 years We have had a few opportunities to examine serum after recovery, the longest interval was 15 years and agglutinins were present in all cases Subclinical infections may cause agglutination reactions of 1/30 or 1/100 as Alston and Brown (1935) found in 9 out of 20 sewermen who had no

state With the passage of time the nonspecific co agglutinins disappear before any demonstrable diminution occurs in the titre of the specific antibodies

The production of the nonspecific co agglutinins cannot be ascribed to any one common factor If a single strain of

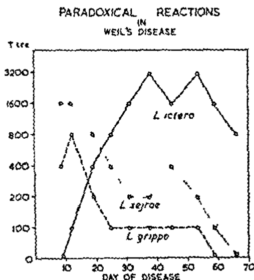


Fig 35

Paradoxical reactions in Weil s disease By Dr F Fühner Adapted from Zeitschrift für Immunitätsforschung und exper mentelle Therapie by kind permission

leptospires constantly evoked the same heterologous antibodies in any one host species it could be assumed that leptospires contain ' potential antigens which act as complete antigens in some host species but not in others Such however is not the case, either in natural infections in man, or in guineapigs immunized against various serotypes

Wiesmann considered that the production of these antibodies can most readily be explained if one assumes that the antibodies to different serotypes are very similar in chemical composition As the result of changes in the host (perhaps variation in the serum globulin fractions) brought about by the disease process,

history of having had an attack of jaundice Protective antibodies were found in these sera by protection experiments in guineapigs

The possibility of past infection is important in patients who have been in contact directly or indirectly with rats McKeon and Brown (1936) described an attack of infective hepatitis in a miner, where the diagnosis was complicated by a positive leptospiral agglutination We have records of three similar cases The patients, a farm worker, a seaman and a refuse collector, all suffered from jaundice Each case showed a positive agglutination titre of 1/100 or 1/300 but, as there was no increase at subsequent tests, the illness was obviously not Weil's disease

CO-AGGLUTINATION —Serum from a case of leptospirosis frequently reacts positively with two or more serotypes, and Wiesmann (1952) considered the co-agglutinins responsible for this effect to be of two types, which he designated respectively as specific and nonspecific

The presence of specific co-agglutinins can be explained on the extant theory that leptospires (like other organisms) contain a number of antigenic factors each of which elicits its corresponding antibody When two or more serotypes (*e g* members of the Hebdomadis serogroup) contain a factor in common, the antiserum of each will agglutinate the heterologous serotypes The titre of an antiserum for the heterologous serotypes will of course depend on the concentration of the co-agglutinins, and this in turn would presumably be determined by the relative amounts of the common antigenic factor present in the different serotypes In these cases cross-absorption tests are necessary to determine the identity of the serotype which caused the infection

On the other hand the nonspecific co agglutinins do not correspond to any antigenic fraction of the homologous serotype, and they are not invariably *present They may* agglutinate one or more heterologous serotypes and furthermore the sera of different individuals infected with the same strain may not *all react with the same range of serotypes* During the course of the illness the homologous antibodies increase, whereas the nonspecific co agglutinins diminish and *finally disappear The impermanence* of their nature can be noted also in immune sera which have been stored in the fluid

CHAPTER XIII

TREATMENT

The most important lesion in leptospiral infection is renal damage, and specific antileptospiral serum and antibiotic drugs to which leptospires are sensitive cannot usually be given early enough in the illness to protect the kidney from serious damage by the more virulent serotypes In this Chapter therefore the details of the treatment of renal failure are described first, followed by a brief account of treatment by antibiotic drugs which may be considered useful when there is ground for suspecting that an illness of only 1 to 3 days' duration is of leptospiral origin Finally an account is given of experimental and clinical experience of the effect of antiserum and antibiotic drugs

TREATMENT OF RENAL FAILURE

All serotypes which have been tested are sensitive to penicillin to about the same extent as bacteria such as the Oxford staphylococcus Some degree of benefit may be obtained by antibiotic treatment at any stage of infection caused by a sensitive pyogenic organism On the other hand, in leptospiral disease a renal disturbance may be started which continues in more or less serious degree whether or not the leptospires in the body are reduced by antiserum, antibiotic drugs or the patient's reaction It would seem therefore that there may be an important difference in the disease processes produced by leptospiral and pyogenic infections For this reason there has been—both before and after the advent of antibiotic drugs—an interest in treatment of the renal lesions The forms of treatment proposed have followed those suggested for the similar renal lesions caused by blackwater fever, transfusion of incompatible blood or crushing of muscle These methods have included (1) a régime of low protein diet, with careful attention to fluid balance, glucose by mouth and intravenous

the composition of the antibody might be altered slightly and so give rise to these nonspecific co agglutinins

THE PARADOXICAL REACTION —In the early stages of illness the titre of some of the nonspecific agglutinins may be higher than that of the homologous antibody This Paradoxical Reaction was studied by Fuhner (1950 a) who followed the changing antibody levels during the progress of the disease in a considerable number of human patients The findings in one of Fuhner's cases provided a particularly good example and are shown in Fig 35, where it will be seen that the homologous titre did not gain the ascendency until the fourth week of illness It is therefore necessary to take the stage of illness into consideration when interpreting the results of serological tests In early cases it is advisable to report only that the patient is suffering from leptospirosis, and to complete the diagnosis at a later date when the infecting serotype can be definitely determined

fluid (either water or electrolyte solutions) is recorded and output is entered, specimen by specimen, under the headings of urine, vomit (which is returned by tube to the stomach if Bull's regime is followed), drainage and diarrhoea The results of appropriate analyses of urine and blood are also recorded on the card

2 Collect intravenous blood under paraffin for estimation of sodium potassium, chloride, bicarbonate and urea

3 Correct any imbalance of electrolytes by intravenous injections and give sodium lactate intravenously to counteract acidosis

4 If the concentration of potassium in the blood is too high, correction can be made by giving a cation exchange resin by mouth, by giving glucose and insulin together intravenously, or by a combination of both methods

5 For the application of Bull's regime or modifications of it, pass a plastic tube, of 2 to 3 mm bore without a bulbous tip, into the stomach Drip into the tube, steadily during each 24 hours, 400 g of glucose dissolved in water to a total volume of 1 litre * Collect all the patient's vomit, filter it through lint and return it to the stomach by the tube If vomiting is frequent, rapid absorption of the glucose may be achieved by injecting a 45 per cent solution in water into the superior vena cava, by means of a polythene tube passed into either of the median basilic veins When the urinary output reaches one litre per day, stop feeding by stomach tube and give a diet low in protein with a quantity of water equal to the volume of urinary output in the previous 24 hours, augmented by an amount estimated to replace the loss per day by perspiration and respiration This may be taken to be about 500 ml in temperate climates, and a litre or more in hot humid countries

If treatment by intravenous glucose is not successful, recourse should be had to the 'artificial kidney' The indications for its use are either the onset of uraemic coma, or a rise of the blood K to more than 8 m Eq per litre and a fall of the HCO_3 to less than 10 m Eq per litre

* Bull's original prescription was Glucose 400 g peanut oil 100 g acacia q s to emulsify vitamins optionally water to 1 litre This fluid has a milky appearance and looks more nutritious than the watery glucose solution but many clinicians do not use the oil now, and especially avoid it when vomiting might cause it to enter the lungs

drip treatment with saline or glucose saline, (2) splanchnic block or high spinal anaesthesia (Robertson, 1946, Williams 1947), (3) a form of conservative treatment of anuric uraemia described by Bull, Joekes and Lowe (1949) and by Bull (1955) The success of the first two methods has not been proved by controlled use in severe leptospiral infections and the third is at present considered the most useful in cases of oliguria and anuria from most causes (Merrill, 1955)

A critical trial of the method in leptospirosis has not yet been reported, but Trimble (1954, 1957) stated that it was used in a number of severe cases in Malaya and he believed that it was responsible for the low death rate of 2 per cent in his series At present this method appears to be the most advantageous in the treatment of cases with severe renal involvement

Bull (1955) summarized the aims of treatment of uraemia from any cause as follows

Adjust intake and rate of endogenous production of chemical substances to balance their rates of excretion when the kidneys are working at their maximum efficiency

(1) Improve renal function
- (a) Specific therapy depending on the cause
- (b) Correct circulatory renal insufficiency

(2) Adjust intake and rate of production of substances
- (a) Reduce katabolism
  - (i) Combat infection
  - (ii) Hormone therapy
  - (iii) Protein-sparing diet
- (b) Adjust intake of H_2O, Na, K, Cl and N
  - (i) To correct imbalance
  - (ii) To maintain balance

(3) Treatment of symptoms
Anaemia, nausea and vomiting, stomatitis, diarrhoea, convulsions, insomnia delirium hypertension

The practical application of this outline to severe leptospiral infections with renal failure may be made as follows

1 Chart the patient's intake and output of fluids This must be done accurately and systematically for successive periods of 12 to 24 hours, and may be recorded on a form such as that produced by Preedy and Richardson (1956) The intake of

Weil s disease by subcutaneous and intravenous injection reduced the case mortality from 30 to 17 per cent, but the experiment was not well controlled and the results are not convincing Walch-Sorgdrager (1939) analysed a series of 48 patients treated with serum, and compared them with about 100 who did not have serum She did not compare the death rates in the two groups, but found that relapses were rather more frequent with serum than without it and that the duration of the febrile period was the same in the two groups From observation of a few very acute early cases she believed that serum was beneficial when it could be given during the first four or five days of illness

Robertson (1946) reported his experience of 12 cases which received specific antileptospiral serum during the first four days of illness None of these patients died whereas there was a 30 per cent death rate among about 20 others whose serum treatment could not be begun before the fourth day [It is not made clear whether these 20 patients received serum after the fourth day or not at all] Although the results suggest that serum may have had a decisive effect in the early cases, it is possible that the patients who came to hospital early were mild cases detected by practitioners on the lookout for the disease Some experiences of our own were of the same indecisive nature In a series of 58 sewer men treated in London, 13 received serum , of these 2 died (15 per cent) while the case mortality rate for the other 45 was 22 per cent It is almost certain that the serum was given to the more seriously ill patients in this group In recent years the use of antiserum in therapy became superseded by the endeavour to find a more effective treatment by antibiotic drugs

Serum Treatment of Experimental Disease in Animals

Inada *et al* (1916) showed that guineapigs could be protected against infection by *L icterohaemorrhagiae* if immune serum were injected at the same time as the leptospires and this finding has often been confirmed For instance Larson and Griffitts (1945) found that 95 per cent of white mice could be protected against infection if a single dose of serum was given within 48 hours of infection, but with decreasing efficacy later Das Gupta (1939 d) protected guineapigs against subsequent in-

The aim of treatment is to nourish the patient, maintain the electrolyte balance and avoid as much as possible the metabolism of protein while the kidneys are not functioning Recovery of the kidneys is independent of this form of treatment, but when recovery occurs secretion of urine may quickly return and a litre may be produced during the first day

There does not appear to be any specific form of treatment available to hasten the return of the liver to normal functioning and there is no evidence that any permanent harm is done to this organ by leptospiral infection

TREATMENT BY ANTIBIOTIC DRUGS

Analysis has shown that treatment by antibiotic drugs does not benefit patients suffering from leptospiral infections when—as is usual—the treatment is not begun before the third or fourth day This conclusion refers both to the more severe forms of leptospirosis such as Weil's disease (Broom, 1951 a) and the milder forms (Fairburn and Semple, 1956) However, if it is suspected that a very recent illness of one to three days' duration is leptospiral, penicillin may be given intramuscularly in dosage of 600,000 units 6-hourly for at least 5 days, or longer if necessary until the patient has been afebrile and free of symptoms for 24 hours Babudieri (personal communication) claims good results when penicillin therapy is begun at the first appearance of symptoms in suspected cases of ricefield fever As an alternative, chlortetracycline intramuscularly 0 5 g 6-hourly or oxytetracycline orally 0 5 g 6 hourly may be used for a similar period

RECORDS OF TREATMENT OF HUMAN INFECTIONS AND OF EXPERIMENTAL INFECTIONS IN ANIMALS BY ANTISERUM AND BY ANTIBIOTIC DRUGS

Serum Treatment of Human Disease

Although treatment of Weil's disease by antileptospiral serum was often used before antibiotics were available, and good results were recorded, *there is no extensive* controlled test of its effect Inada (1922) considered that serum treatment of

the summer of 1944. He states that 39 cases of Weil's disease were diagnosed, but he gives no details of the number treated with penicillin or of the results obtained. He formed the impression, however, that the antibiotic had a useful therapeutic action. On the other hand, Smith (1949) from the experience of 16 cases, thought that penicillin had little effect in toxic patients.

As compared with these small numbers, we have records of 206 cases of Weil's disease treated with penicillin in a number of hospitals in Britain during the years 1947-50 inclusive. Details of dosage and of the day of disease on which treatment was begun are, however, available for a minority only. Thirty-four of these patients died. This represents a case mortality of approximately 17 per cent, whereas the rate for the whole series was 15 per cent. From the crude figures it thus appears that penicillin therapy does not improve the prognosis of Weil's disease.

TABLE XXIV

WEIL'S DISEASE. DEATH RATES FOR JAUNDICED CASES (Broom 1951 a)

Description	*No of Cases*	*Deaths*
Cases in 1947–50	263	54 (20%)
Cases treated with penicillin	152	34 (22%)
Cases in 1940–6	103	24 (23%)

To compare these results with the position before penicillin was available the series reported by Broom and Alston (1948) may be used. None of the patients in the latter series received penicillin, and the death rate was 22 per cent. It must be noted, however that the proportion of non icteric cases was also much lower, and as noted above, death is very rare in the absence of jaundice. It therefore seemed that a fairer comparison would be the death rates among jaundiced patients. Table XXIV shows that the corrected case mortality for the earlier series now becomes 23 per cent. For all jaundiced cases in the present series the rate is 20 per cent, for those treated with penicillin it is 22 per cent. When the crude statistics are corrected in this manner there is still no indication of any benefit from treatment with penicillin.

These results may be analysed in another way. From the animal experiments it was evident that the curative effect of penicillin was

fection for 25 and not more than 27 days by human immune serum, for 41 and not more than 52 days by rabbit immune serum, and for 10 days by therapeutic horse serum

Uhlenhuth, Schoenherr and Zimmermann (1950) protected hamsters against infection with *L canicola* by injection of the serum of dogs which had recovered from natural infection with either *L canicola* or *L icterohaemorrhagiae* In an experiment to test the duration of passive immunity, hamsters were inoculated subcutaneously with 0 5 ml of this serum Groups of hamsters were challenged with *L canicola* after 2, 3, 4, 5 7, 8, 9, 19 or 30 days and all the animals survived When sacrificed from 4 to 6 weeks later, the hamsters in the groups up to and including 9 days, showed no abnormalities in the kidneys and no leptospires in the organs With one exception, the kidneys of the animals in the last two groups all showed macroscopical evidence of scarring, and leptospires were present in considerable numbers

Gadeke and Schoenherr (1952) infected young hamsters with a *virulent strain of L canicola* which *killed untreated* animals in an average time of 79 hours Hamsters which were similarly infected and treated after 24 hours with immune serum were *killed at different periods afterwards Those killed* 112 hours after treatment showed a progressive cirrhosis of the liver and glomerulo nephritis, cicatrization of a number of glomeruli, and commencement of *chronic interstitial nephritis It may* be noted here that McIntyre and Montgomery (1952) found similar renal lesions in dogs which had recovered from infections by *I canicola*

Drug Treatment of Human Disease

Many reports on the use of antibiotic drugs, especially penicillin, have been based on single cases or small groups, as was noted by Broom (1951 a) who analysed the results of treatment of Weil s disease with penicillin He stated

> Not infrequently, the drug was first administered at a comparatively *late stage of illness by which time the outcome of* the attack has already been determined by the extent of the kidney damage, unless other lines of treatment are adopted

Of those observers who have treated a number of cases, Bulmer (1945) was dealing with soldiers who contracted the *disease during*

Queensland, Australia The infecting serotypes were *L australis A* (38 cases), *L australis B* (29), *L canicola* (6), *L hyos* (7), *L icterohaemorrhagiae* (3), *L pomona* (2), *L celledoni* (6), *L mini* (5), Kremastos type (13) and Robinson type (6) None of the patients died, none showed oliguria or definite jaundice, and albuminuria was found in only 23 The patients were divided into six Groups for evaluating the results A, no antibiotics (8 cases), B, penicillin 100,000 units, 3-hourly (25 cases), C, penicillin, in dosage greater than B and less than D (20 cases), D, penicillin 500,000 units, 3 hourly

TABLE XXVI

CLASSIFICATION OF PATIENTS ACCORDING TO SEROTYPES
(Fairburn and Semple 1956)

	No of patients	*Group A (controls)*	*Group B (penicillin)*	*Group C (chloramphenicol)*
SEROTYPES				
L australis A	1	—	1	—
L australis B	3	—	2	1
L bangkinang	2	—	—	2
L canicola	8	3	3	2
L celledoni	1	—	—	1
L grippotyphosa	3	—	—	3
L poi	1	1	—	—
L pomona	1	—	—	1
L wolffii	1	—	—	1
SEROGROUPS				
Australis A	1	—	—	1
Autumnalis	3	—	—	3
Hebdomadis	17	4	8	5
Icterohaemorrhagiae	5	1	1	3
Javanica	2	1	—	1
Pyrogenes	1	1	—	—
Unidentified	33	20	6	7
Total	83	31	21	31

(26 cases), E, chloramphenicol in daily doses from 1 5 to 3 0 g (12 cases), F, chloramphenicol plus penicillin (20 cases) The average duration of the febrile periods of the six Groups (A to F) was 6 2, 5 4, 4 75, 4 3, 6 0 and 5 4 days respectively The average duration of fever in hours, after the beginning of treatment, of Groups B to F was 70, 39 2, 34, 62, and 72

The author pointed out that the duration of illness was lowest

greatest when the drug was given during the first days of illness Suchett Kaye (1951), recording the successful treatment with penicillin of six consecutive cases stresses the importance of the early institution of antibiotic therapy

We have records of 51 cases in which the day of disease on which *treatment* was begun is known, and the results are shown in Table XXV Thirty-six patients recovered, and for the last line of the table the number of recoveries which would be expected in each period if the drug had no beneficial action has been calculated The probability (P) that the *differences could be the result of chance* is 0 8, which means that a difference as great or greater than this would be expected 8 times in 10 trials

TABLE XXV

WEIL'S DISEASE PENICILLIN AT DIFFERENT STAGES
(Broom 1951 a)

	Interval in Days from Onset to Treatment				*Total*
	1–5	6–10	11–15	Over 15	
Deaths	2	11	2	0	15
Recoveries	8	19	5	4	36
Recoveries expected	7 0	21 2	7 8		36

P 0 8

The conclusion must be drawn, therefore that these patients at least obtained no benefit from the administration of *penicillin* at any stage of the disease

If the general case mort [illegible] led to the series reported by Suchett-Kaye [illegible] of every patient in a set o[illegible] occur in approximately 5 out of 10 such sets as the result of chance alone if the treatment had no effect whatsoever

Since this analysis was made there have been no reports which compared the course of infection by *L icterohaemorrhagiae* in patients treated with and without antibiotic drugs

Doherty (1955) collected the results of treatment of 111 patients suffering from leptospirosis, in eight hospitals in

Queensland, Australia The infecting serotypes were *L. australis A* (38 cases), *L. australis B* (29), *L. canicola* (6), *L. hyos* (7), *L. icterohaemorrhagiae* (3), *L. pomona* (2), *L. celledoni* (6), *L. mini* (5), Kremastos type (13) and Robinson type (6) None of the patients died, none showed oliguria or definite jaundice, and albuminuria was found in only 23 The patients were divided into six Groups for evaluating the results A, no antibiotics (8 cases), B, penicillin 100,000 units, 3-hourly (25 cases), C, penicillin, in dosage greater than B and less than D (20 cases), D, penicillin 500,000 units, 3-hourly

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L canicola	8	3	3	2
L celledoni	1	—	—	1
L grippotyphosa	3	—	—	3
L poi	1	1	—	—
L pomona	1	—	—	1
L wolffii	1	—	—	1
SEROGROUPS				
Australis A	1	—	—	1
Autumnalis	3	—	—	3
Hebdomadis	17	4	8	5
Icterohaemorrhagiae	5	1	1	3
Javanica	2	1	—	1
Pyrogenes	1	1	—	—
Unidentified	33	20	6	7
Total	83	31	21	31

(26 cases), E, chloramphenicol in daily doses from 1 5 to 3 0 g (12 cases), F, chloramphenicol plus penicillin (20 cases) The average duration of the febrile periods of the six Groups (A to F) was 6 2, 5 4, 4 75, 4 3, 6 0 and 5 4 days respectively The average duration of fever in hours, after the beginning of treatment, of Groups B to F was 70, 39 2, 34, 62, and 72

The author pointed out that the duration of illness was lowest

in Group D which received most penicillin and was significantly lower, by statistical tests, than in Group B which received least The importance of these results is *somewhat decreased* by the fact that the antibiotic regime, the time of temperature taking and the use of antipyretics differed in the hospitals concerned, and that only 8 patients were given no antibiotics

Fairburn and Semple (1956) analysed the results of treating 83 patients in Malaya with penicillin or chloramphenicol for leptospirosis caused by a variety of serotypes (Table XXVI)

The disease was not severe, only 4 per cent of the patients became jaundiced and 5 per cent had oliguria (*i e*, less than 10 oz of urinary output in 24 hours) or anuria The patients were men aged 18 to 35 and were allotted in rotation to one

TABLE XXVII

AVERAGE TIME FROM ONSET TO FREEDOM FROM SYMPTOMS AND SIGNS
(Fairburn and Semple 1956)

Treatment group	*Symptom free (days)*	*Sign free (days)*	*Apyrexial (days)*	*Symptom free and sign free (days)*
A (*controls*)	9 4	9 9	9 0	10 8
B (penicillin)	8 9	9 5	7 6	9 7
C (chloramphenicol)	8 9	10 0	8 8	10 5

of three Groups Those in Group A (31 patients) received no antibiotics, those in Group B (21) were given 600 000 units of penicillin 6-hourly and those in Group C (31) received 0 5 g of chloramphenicol 6 hourly Treatment was started as soon as the diagnosis was made on clinical evidence Antibiotics were given for at least 5 days and often beyond that time until the patient had been afebrile and free of symptoms and signs for 24 hours The average duration of treatment in Group B was 6 days, and in Group C 6 2 days Treatment in the control Group consisted of rest in bed, fluid replacement and analgesics for pain, this regime was also employed in Groups B and C Symptoms and signs were recorded daily on a standard chart

by one of two observers who were unaware of the Group to which the patient had been allotted Table XXVII shows that there was no significant difference among the three Groups in regard to the average time from onset of illness to freedom from symptoms and signs This was true even in the cases of the three Groups in which treatment was started on or before the fourth day of the illness This is a very good analysis and the conclusions are well supported by the evidence

Clein (1950) disagreed with Fairburn and Semple's conclusions because he believed that 600,000 units of penicillin 4-hourly for 5 days had caused dramatic improvement of symptoms and reduction of pyrexia in all of approximately 24 cases of leptospirosis in Malaya He did not however record the course of illness of any patients who did not receive penicillin, and therefore his statement of belief was very much less convincing than the report with which he disagreed

There have been reports of the supposed benefit, to single cases or small groups, of treatment with chlortetracycline (aureomycin) or oxytetracycline (terramycin) (*e g*, Lurie, 1949, Batchelor and Todd, 1950 Whitehouse, 1952) More convincing evidence however is put forward by Hall, Hightower, Diaz Rivera, Byrne Smadel and Woodward (1951) who in Puerto Rico contrasted 67 patients treated by antibiotic drugs with 12 others to whom such drugs were not given The drugs used were either penicillin streptomycin chlortetracycline, oxytetracycline, combined streptomycin and chlortetracycline, or cortisone and chlortetracycline They concluded that the antibiotics had no appreciable effect, even when given at a relatively early stage of the disease

In summary, the most valuable reports agree that treatment by antibiotic drugs is of little or no value in the severe or the mild forms of leptospiral infection in human beings unless, possibly, the drugs are given as soon as the first symptom appears This agrees with most of the results of treating experimental infections in animals

Drug Treatment of Experimental Disease in Animals

Fairly consistent results have been reported of the results of treating leptospiral infections in guineapigs, hamsters or white mice by antibiotic drugs Success is obtainable only if the

drug is given at the time of experimental infection or soon afterwards—before the appearance of jaundice Heilman and Herrell (1944) and Alston and Broom (1944) found that penicillin prevented the appearance of symptoms in guineapigs provided that treatment was begun within 24 hours of infection with *L icterohaemorrhagiae* These observers and also Augustine Weinman and McAllister (1944) found the drug completely inactive if it was withheld until the animals showed signs of illness Larson and Griffitts (1945) saved 95 per cent of infected animals when 50 to 100 units of penicillin were given within 48 hours of infection, but only 28 per cent when the drug was delayed to 88 hours However, Borg Petersen and Schmidt (1945) were able to cure the disease in guineapigs even when treatment was not begun until the fourth day after infection by which time the animals were clearly sick Chang (1946) concluded that penicillin seemed to have some therapeutic effect in infected guineapigs if given before the appearance of jaundice but not later

Successful results have been obtained in treating hamsters infected by *L icterohaemorrhagiae* with streptomycin 17 hours after inoculation (Heilman, Knutson and Greenburg, 1945) and in treating the same animals infected with *L canicola* by chlortetracycline and oxytetracycline but not by chloramphenicol (Uhlenhuth and Schoenherr, 1951) Gadeke and Schoenherr (1952) found that young hamsters infected by *L canicola* and treated successfully by oxytetracycline showed no lesions in the liver There was some residual scar tissue in the kidneys, but not the progressive chronic nephritis which occurred in animals treated by immune serum

In experiments in guineapigs and in hamsters Faine and Kaipainen (1955) found that erythromycin could prevent the death of the animals if the drug was given from the first day after infection, but a high proportion of the animals died between the seventh and thirteenth days from toxic effects of the drug The drug was similarly toxic to guineapigs which had not been infected

CHAPTER XIV

GENERAL PREVENTION AND PERSONAL PROPHYLAXIS

In planning schemes for the prophylaxis of leptospirosis three factors must be considered (1) the oecology of the carrier host, (2) the mode of transmission of infection and (3) the population at risk As each of these factors is variable for the different circumstances of infection, the conditions which may obtain in any outbreak are almost unlimited Certain broad principles that govern prevention may be generally applicable, but no hard and fast rules can be laid down A good illustration of this is given by the sex incidence of sejroe fever in Denmark, already referred to (p 77), first, men were infected while harvesting in the fields, and later, when the carrier mice left the fields for the farm buildings, women were infected

Another example is provided by Weil's disease contracted while bathing These cases can be divided into two groups, depending on whether the patients have been bathing in official swimming pools, or indiscriminately in stagnant ponds and streams In the first instance, it would be the duty of the responsible authority to cleanse the pool, remove any debris likely to attract rats, and to prevent them as far as possible from gaining access to the water When these precautions were adopted in the Netherlands, Walch-Sorgdrager (1939) reported that bathing infections virtually ceased Such measures are not possible when bathers make use of any available stretch of water, and prevention can only be achieved by making swimmers aware of the risk Boys and young men are the most frequent sufferers, so that warnings issued in schools and Service establishments might be effective Simple prohibition may be insufficient, as was shown by the outbreak of the so called seven-day fever, due to *L hebdomadis*, among U S troops who had bathed in 'off-limit' ponds on the island of Okinawa, reported by Gauld *et al* (1952) Special efforts should be made during hot summers when the temptation to engage in casual

bathing is greatest, for example Broom (1951 a) noted that 33 cases occurred in England in the hot summer of 1949 in contrast with only 15 in 1948 and 16 in 1950 when the summers were cool

The preventive measures that might be applied according to circumstances include

1 Destruction of rodents
2 Rodent proofing of buildings
3 Hygiene of premises and land, food protection
4 Isolation, destruction vaccination or drug treatment of infected domestic animals
5 Hygiene of human individuals
6 Warning to doctors of the risk of infection in certain occupations, and visiting men absent from work
7 Immunization passively by serum or actively by vaccine
8 Prophylactic use of Penicillin V

DESTRUCTION OF RODENTS

An extensive investigation of methods for the control of rats and mice was carried out in England from 1939 onwards and a summary of the work was published by Chitty and Southern (1954) The studies were designed primarily to determine the best means of controlling *R norvegicus* *R rattus* and *M musculus* in rural and urban areas (including sewers) in this country, but experiments were also made by Watson and Perry (1954) in Palestine and the Sudan Doty (1945) described the methods used on sugar plantations in Hawaii and Harrison and Woodville (1948) carried out a rat destruction campaign in the City of Rangoon Burma

Trapping

All these workers considered that trapping is quite ineffective as a means of controlling heavy infestations, although it may be used successfully in dealing with small numbers of rats, and in disposing of a few survivors after poison treatment The break back treadle type of trap gives the best results, and a large number should be used relative to the size of the infestation The traps should be placed with the treadle at right angles to the runway and they should be left baited but unset for several

days After the rats have grown accustomed to the traps they should be baited and set, and thereafter should be inspected and reset frequently

POISONING

As compared with trapping, a high level of destruction can be achieved by poisoning The exact procedure to be adopted varies according to local conditions at the site of infestation Success depends on planning and carrying out the campaign in accordance with certain general principles which have been established on the basis of observation and experiment, and which have been tested and proved in field practice Useful practical details of the methods are given in a pamphlet issued by the Ministry of Agriculture and Fisheries (1953)

The main aim is to place poisoned baits in such a way that the rats will find them on the way from their day-time resting places to their normal night-time feeding grounds This ensures that the maximum number of rats will eat the bait while they are hungry, and are likely therefore to consume a lethal dose A preliminary survey should therefore be carried out to determine the number and position of the sites where bait should be laid If there is doubt about the presence of rats test baits (not poisoned) should be laid

Rats, especially *R norvegicus*, are very suspicious of new objects introduced into their environment, and avoid them until they become familiar It may however be necessary to place bait in receptacles, either to prevent the risk of poisoning man, domestic animals or birds, or to protect the bait from the effects of weather, etc All such objects should be placed in position 10 to 14 days before baiting is begun, and they should not be moved during the whole period

It is equally important to get the rats accustomed to feed while they are hungry on particular baits at chosen sites This is achieved by 'pre-baiting' usually on two occasions with the same bait which will later act as a vehicle for the poison When the '1-3-5' system is employed pre-baiting is carried out on days 1 and 3, and poisoning on day 5 Uneaten poison bait is removed next day, and any surviving rats should be left undisturbed for 2 weeks to allow them to resume normal feeding habits Then test baits are laid at all the points

previously poisoned Where bait is taken by the third day, the sequence of pre-baiting and poisoning is repeated at these points only Rats which survive after a poison treatment may have consumed a sublethal dose of poison, and are likely to develop 'bait-shyness' and refuse to take the same bait again For second and subsequent poisonings therefore different baits and different poisons must be used The particular foodstuffs which provide baits most acceptable to rats is not the same in all countries Where information on this subject is not available *preliminary tests should be made to determine the most* suitable bait

The methods outlined above were developed for use with poisons such as zinc phosphide, arsenious oxide, red squill and Antu (α-naphthylthiourea) all of which give rise to bait-shyness The discovery that Warfarin (3-α-acetonylbenzyl-4-hydroxy coumarin) is toxic to rodents has provided a new approach to rodent control This substance differs from the other poisons in two important respects (1) it kills only when consumed *repeatedly, and (2) it does not give rise to bait-shyness* There is therefore no need for pre-baiting

Warfarin is a white powder insoluble in water, and when ingested regularly in small doses it leads to fatal internal haemorrhages Baits should be left in place for 1 to 3 weeks according to the rate of consumption, more bait being added as required Only a low concentration is needed to kill rats 0 005 per cent for the common rat and 0 025 for the ship rat Its use therefore considerably lessens the risk of accidental poisoning of other animals, but Clark (1954) records an instance when five piglets died after eating rat bait which contained Warfarin Covered containers are therefore necessary as a safety precaution, and also to protect the baits from the weather and to prevent their being scattered

FUMIGATION

In certain conditions, as where mice are infesting sacks of grain stored in granaries or warehouses, fumigation is the most effective means of destruction Gas proof fabrics are used to cover the stacks, and the fumigant (*usually methyl bromide or* hydrogen cyanide) is introduced underneath Special regulations have to be complied with when cyanide gas is used inside

a building Gassing is also an effective method of destroying rats in banks and hedgerows which are not near inhabited buildings Powders which release hydrogen cyanide are pumped into one of the holes, and pumping is continued until powder is seen emerging from other holes These are immediately sealed and the process continued until no further holes are revealed An alternative method is to place a teaspoonful of powder some distance down the holes, and then seal them

RODENT PROOFING OF BUILDINGS

There are so many differences in type of building materials and in the purposes for which buildings are intended that it is not possible to give more than the general principles involved in denying rats access to premises A useful summary of the methods used in practice is given by the Ministry of Agriculture and Fisheries (1953)

Even before a building is erected rodents may be present on the site and must be eradicated in case they may infest the building during construction Surface cover such as heaps of refuse should be removed or destroyed Where possible disused [illegible]

to ensure that all service pipes are properly built-in where they pass through the outside walls, and pipes fixed to the outside of the building should be protected by a metal collar to prevent external climbing Ventilation grids and airbricks should not have single openings larger than one quarter inch in diameter Doors and windows should fit closely, and projecting wooden surfaces which rats could gnaw should be avoided External [illegible]

wells should be so constructed that rodents cannot contaminate the water

HYGIENE OF PREMISES AND LAND, FOOD PROTECTION

Hygiene in industrial premises and at sites of outdoor work, in farms and fields and in the home has a wide application in countering many types of leptospiral infection In one broad aspect, it includes removing edible refuse and water in drinkable quantities from buildings or working places, and swabbing floors and benches with antiseptic, if possible Ido *et al* (1916) considered that by disinfecting the ground and by removing stagnant water in certain places in coal mines in Japan they had twice prevented epidemics of Weil's disease

Davidson *et al* (1934) investigated unhygienic conditions in the cleaning of fish in Aberdeen, Scotland, and demonstrated *L icterohaemorrhagiae* in floor washings and in water contained in tubs under the work tables From the results of experimental work, Davidson and Smith (1936) recommended that floors, benches and fish boxes should be thoroughly hosed down and that all debris should be removed each evening Half an hour before work begins in the morning hosing should be repeated, and the benches etc. washed with a solution of sodium hypochlorite Davidson and Smith found 'Chloros' (a proprietary preparation of sodium hypochlorite) very suitable for this purpose This preparation killed *L icterohaemorrhagiae, in vitro,* in a dilution of 1/4,000 when it contained 17 2 parts per million of residual chlorine The manufacturers recommend its use in the strength of two fluid ounces to each gallon of water, giving 1,250 parts per million of available chlorine, this great excess provides for effective disinfection even in the presence of much protein

Kathe and Engelhardt (1953) found that offal from a fish-cleaning establishment in Germany was dumped on the fields around the township and greatly encouraged rats They recommended better hygiene in the disposal of this refuse to help to reduce the high incidence of Weil's disease found among the fish workers In 1948, Molner *et al* investigated conditions in a poultry-dressing establishment in Detroit, U S A, where 18 cases of Weil's disease had occurred The premises were heavily infested with rats, and the work tables were incompletely cleansed of blood and offal at night-time Strains of *L ictero-*

haemorrhagiae were isolated by washing the tables with saline in the morning, but when the tables were swabbed with dilute hydrochloric acid in the evening the leptospires were no longer detected next day In London sewers brickwork of the floors and walls has been made safer by diverting the stream of water to one side by a dam and washing the brickwork with antiseptics (such as 'Chloros') before it is chiselled and removed

In the fields various means have been used in addition to those already described for killing rodents The destruction of leptospires by disinfectant agents has been attempted in appropriate circumstances Tohyama (1927) applied calcium cyanamide as a fertilizer to certain of the rice fields in a part of Japan where Weil s disease was very common As a result the incidence was much reduced both in comparison with the adjoining villages and with the same villages in previous years Taylor and Goyle (1931) found that this fertilizer is strongly alkaline and they believed that its effect is probably due to the toxic action of a polymer of calcium cyanamide (dicyandiamide) which is always present in the fertilizer They noted that the ammonia which is liberated when calcium cyanamide is mixed with water is harmful to most seedlings but not to rice seedlings and indeed Tohyama recorded that the yield of rice was greatly increased by the treatment

Conditions of human infection in rice fields vary from one part of the world to another In Italy Mino (1942 a) showed that the greatest danger is due to invasion of the growing rice by mice They build nests among the plants above the flooded ground and as a result they and their young may heavily contaminate the plants and water with various serotypes of leptospires This gives rise to a situation which is difficult to counteract According to Altava *et al* (1953) Babudieri failed in attempts to reduce leptospiral infections in the Italian rice fields by using calcium hypochlorite salts of copper and change of *p*H In the province of Castellón in Spain Altava *et al.* showed that the danger during harvest was due to the fact that the ground was dry at that season and that rice plants were *contaminated by the urine of rats which came into* the fields in the early morning from the surrounding canal banks Here, infection could be counteracted by cementing the canal banks

and by allowing the fields to remain flooded until harvesting was over

Johnson (1939, 1950) stated that burning the dry leaves at the base of sugar canes immediately before they are cut has been found effective in reducing infection of cane cutters in low lying districts of North Queensland, Australia The infections are mainly caused by either *L australis A* or *L australis B* carried by ***Rattus conatus*** and to a lesser extent by *R rattus* The burning has no ill effects on the cane and it drives out the rats evaporates rat urine and small accumulations of surface water and removes the leaves and 'trash According to Doherty *et al* (1956) the following provisions for the prevention of leptospirosis in North Queensland are laid down in 'The Rat Prevention and Destruction Regulations' of 1942

1 Cutters are encouraged (but cannot be compelled) to wear protective clothing especially adequate boots This measure is not popular with cutters and few would receive any protection against leptospirosis from their clothing

2 Farmers are compelled to keep all garbage and waste in such a way that it does not furnish food for rats or bandicoots They are also compelled to keep down undergrowth and grass that may provide rat harbourage

3 Canefields are inspected before cutting A block that is considered to carry a risk of leptospirosis, either by the extent of rat damage or due to the ground conditions (in the words of the Act 'where such field is low lying badly drained or wet) is placed under a Health Order This Order forbids cutting until the cane has been effectively burnt The criteria of an effective burn as set out in the Act are that the cane should be burnt 'so as to remove or effectively mitigate to the satisfaction of an Inspector the hazards which have arisen or are likely to arise from rats mice or bandicoot infestation'

Sawers (1938) noted that the heat generated by burning the 'trash' did not appreciably penetrate into the soil, although it was sufficient to destroy leptospires on the cane and the surface of the ground This is an important fact because Smith and Self (1955) showed experimentally that *L australis A* could survive for some weeks in artificially infected soil and that th

leptospires were washed out when the soil was flooded with rain water These findings may explain why cane cutters frequently become infected when heavy rain has followed effective burning

For the infections which occur during the harvesting of various other crops some means of prophylaxis has yet to be found. Litzner and Hahn (1950) and Popp (1950) described an explosive outbreak of infection by *L grippotyphosa* among pea harvesters Similarly, Hermannsen (1954) reported that in 1952 in Schleswig-Holstein, three hundred people were infected by the same serotype while picking peas during August or gathering cabbages during October and November These outbreaks were caused by contamination of the plants by the urine of very large numbers of *Microtus arvalis* With rare exceptions, the patients had handled the plants while they were soaked with dew, and it was believed that the voles had contaminated the plants in the early hours of the morning Neither burning nor the use of antiseptics on the [illegible] l or on the plants would be applicab[illegible] but if harvesting [illegible] plants and kill[illegible] [illegible]spires, infections might be reduced [illegible]vesting must be done by hand, leather gloves would provide good protection and probably cause very little hindrance in the work of gathering the vegetables

In farm yards and buildings where animals are kept, hygienic conditions are necessary as well as isolation of animals known to be excreting leptospires In Australia, Israel and the U S A, *L pomona* and *L grippotyphosa* have been found capable of infecting large numbers of cattle and of spreading within a herd of cattle, or from pigs to cattle or man In New Zealand, Kirschner *et al.* (1952) recorded the case of a boy aged nine who was in the habit of walking barefoot about a farmyard where there were infected cows

In all premises where food is kept, protection from contamination by animals, especially rodents, is an obvious part of the prevention of leptospirosis In addition to the risk of human infection by the alimentary tract, which is not a high probability, there is danger in handling contaminated food and food containers.

after it was contaminated In Switzerland, Gsell reported a great reduction in incidence when swineherds regularly wore waterproof boots Walking barefoot should be avoided in all farmyards, and on the muddy banks of ponds and streams

In some types of work or for economic reasons protective boots cannot be supplied to the large numbers of people at risk This is the case in the tropics generally, and in the large numbers of workers in the flooded rice fields of north Italy (Babudieri, Bussinello, Bajocchi, Salvi and Massa, 1955) The protective covering of hands and forearms is not usually practicable for people working in wet conditions Thick rubber or leather gauntlets impede work, make the hands hot, and there is the further danger that infective material may get into the glove by the open end or through punctures If this happens the chance of infection may be even increased because the material may be kept in close contact with moist skin. Leather gloves might be useful in some field work where conditions are relatively dry For instance, pea gathering in the fields might be made safer in this way although gloves would be a greater hindrance when stripping the peas from the pods

In the case of infected dogs in the house it should be possible to avoid contact with infective urine if mopping up is done with large cloths soaked with disinfectant In kennels and farm premises protective or working clothes should be removed after work, and hands and forearms washed

The use of barrier creams, impregnated with a compound which slowly gives off chlorine in contact with water, would in theory provide an additional safeguard in many of these situations Unfortunately, the results of the few experiments made by Broom (1951 a) did not indicate that such preparations provide any protection against leptospirosis However, exhaustive trials were not carried out, and more extensive investigations might bring to light an effective compound

WARNING DOCTORS OF THE RISK OF INFECTION IN CERTAIN OCCUPATIONS, AND VISITING MEN ABSENT FROM WORK

In London many employers of sewer men give each employee a card which the man is requested to show to his doctor when

he first becomes a sewer man and whenever he needs medical attention The card warns the doctor of the risk of infection by *L icterohaemorrhagiae*, outlines the symptoms and signs of the disease and names a hospital pathologist who will be willing to do relevant tests and to consult with the doctor if required This plan has produced useful collaboration and has not been resented to a noticeable degree by general practitioners In addition, some employers send a representative to an employee's house as soon as any illness is notified to make sure that a doctor is in attendance and has seen the card

IMMUNIZATION PASSIVE BY SERUM OR ACTIVE BY VACCINE

Passive

When it is thought probable that infection by *L icterohaemorrhagiae* has occurred injections of leptospiral antiserum would seem to be a reasonable precaution on several days of the possible incubation period We are not aware that this has been done in the field, and an alternative or additional treatment might be the prophylactic use of oral Penicillin V, as will be mentioned later

Active

A safe and effective vaccine against Weil s disease in human beings has been sought since the causative organism was first found, but efforts have not been successful so far In this section only the methods of making vaccines for human use, and the immunization of experimental animals on which the methods are based will be described

In the early experiments difficulties arose because, if the vaccine were given subcutaneously, inconveniently large volumes were necessary to produce effective immunity, as judged by the presence of protective antibodies in the serum The vaccine had to be injected intravenously or intramuscularly if smaller quantities were to be used, and by any route of injection undesirable general reactions were liable to occur

Ido *et al* (1916) showed that guineapigs could be immunized by intraperitoneal injections of *L icterohaemorrhagiae* killed by phenol, and also that injections of 0 5, 1 0 and 2 0 ml of

L icterohaemorrhagiae when they are treated with nonvirulent living vaccines, or with formalinized, heat-killed, phenolized and 'Dettolized' suspensions of virulent cultures Vaccination with homologous strains of *L icterohaemorrhagiae* gave better immunity than with heterologous serotypes such as *L hebdomadis* or *L autumnalis* He found that immune bodies were demonstrable in the serum of guineapigs vaccinated with *L icterohaemorrhagiae*, and a lytic titre of 1/6 seemed to indicate that sufficient protective antibodies were present Smith also employed killed vaccines from nonvirulent and virulent strains of *L icterohaemorrhagiae* in experiments on human volunteers The vaccines given subcutaneously in doses of 1 and 2 ml caused little local reaction and no general disturbance, but the amount of antibody response was disappointingly small

Esseveld (1937) reported that leptospires killed by heat, and even by boiling, produced antibodies in rabbits as effectively as living leptospires Human beings were actively immunized by means of virulent strains of *L icterohaemorrhagiae* killed by heating to 70°C for half an hour on two successive days Subcutaneous injection of 2 ml followed by 3 ml of this vaccine produced antibodies to titres up to 1/300 Intravenous injection of 3 ml following the subcutaneous injections, or following an intravenous injection of 1 ml, raised the titre to 1/1,000 or higher Esseveld noted that it would be necessary to determine how long these antibodies persisted

Borg-Petersen and Errebo Knudsen (1953) reported from Denmark the results of vaccinating 30 volunteers, mainly sewer workers They used a concentrated suspension of virulent *L icterohaemorrhagiae* killed by twice being heated to 70°C for half an hour Three subcutaneous injections produced an agglutination-lysis titre of 1/100 in the serum of two men, but one year later the reactions had become negative Twenty-eight men were vaccinated by 2 or 3 (exceptionally 4 or 5) intravenous injections, 15 of the men had troublesome generalized clinical effects after at least one or other of the injections, and 6 were unfit for work for one to several days

The antibody response to intravenous injection was good especially when a stimulating dose was given a year after the first injections In those who received only the two basic injections, agglutinin titres of 1/10 to 1/100 were still present

three years later in 6 out of 9 persons, and the sera of 3 protected guineapigs against infection In the group which received the additional stimulating inoculations, agglutinins of the same order were demonstrable three years later in 12 out of 13, and the sera of 8 contained protective antibodies The authors concluded that although the vaccine was shown to be effective the severe clinical reactions were too frequent to warrant its general use

By contrast Babudieri *et al* (1955) considered that they obtained a high degree of protection by subcutaneous injection of a mixed vaccine of *L icterohaemorrhagiae* and *L bataviae* In preparing the vaccine, the organisms were killed by the addition of 0 3 per cent formalin, and were sedimented by centrifugation To remove all traces of serum which might produce allergic reactions, the sediment was washed twice and resuspended in formol saline to one-tenth the original volume of culture The vaccine was then made by mixing 2 volumes of *L icterohaemorrhagiae* suspension with 1 of *L bataviae*, and was administered in doses of 1 ml The subjects of the experiment were 303 women, all of whom were starting work in the Italian rice fields and so had had no opportunity of being infected previously About half received a single dose of vaccine, and the remainder were given a second dose one week after the first The formalin in the vaccine caused a stinging sensation which soon passed off, but otherwise there were no reactions either local or general Agglutination tests made on sera of 85 of the women showed wide variations in the response to vaccination, but not one of the 303 vaccinated persons developed leptospirosis during the year 1954 In a control group of 202, also newcomers to the rice fields, there were 116 cases during the same period A *L icterohaemorrhagiae* vaccine prepared by the same method was used by Altava, Barrera, Villalonga, Gil, Marin and Babudieri (1955) in Spain to inoculate 72 ricefield workers whose blood contained no antibodies During the next harvest season no cases of Weil's disease occurred among these workers, whereas there were 3 cases among a control group of 79 unvaccinated negative reactors, and a further 13 among 1,000 other unvaccinated workers whose agglutination reactions had not been tested

PROPHYLACTIC USE OF PENICILLIN V

It has been suggested above that Penicillin V might be given orally to workers such as sewer men who are exposed to leptospiral infection when they suffer cuts or abrasions The same treatment might be given on the first day of suspicious illnesses to workers (such as ricefield workers and pig farmers) in districts where the disease is common The course might consist of 1,000,000 units daily for ten days, given in 3 doses of *200,000* units during the day and a single dose of *400,000* units in the evening

The failure of a short course of penicillin to prevent the disease was reported by Broom and Norris (1957) A laboratory worker whose foot was pricked by a contaminated syringe was given during three days a total of 6 million units of penicillin by mouth, the first dose being taken within an hour of the accident On the tenth day however he fell ill, and the diagnosis was confirmed by isolating the infecting strain by blood culture

CHAPTER XV

LEGAL ASPECTS IN ENGLAND AND WALES OF THE CONTRACTION OF LEPTOSPIRAL DISEASES

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When considering any legal question relating to the administration of law in England it is always important to remember that, *unlike many Continental Countries English law has never* been codified It springs from two principal sources Statute Law and Common Law

Statute Law is the law laid down in Acts of Parliament and also Regulations made under powers given by any particular Act of Parliament One Act of Parliament can repeal another earlier one in whole or in part Thus a constant change is taking place the whole time in Statute Law in an effort to keep it abreast of modern thought and necessity

Common Law is the traditional law of the Country which is to be found explained and developed, in the judgments of the Judges in the Courts, and which has the same force as Statute Law These judgments are to be found in the various Law Reports As a Judge is obliged to follow the earlier decision of a Court superior to his, and tends to follow so far as possible the earlier decisions of Judges of equal rank to himself, such judgments are frequently the law applied in a particular case The highest legal tribunal is the House of Lords and once a decision on a matter of law is given in that place, then that decision remains the law unless the law is subsequently altered by an Act of Parliament

The difference between Statute Law and Common Law will be appreciated in the remainder of this Chapter where in some

parts of the Chapter reference is made to various Acts of Parliament and Regulations, and in other parts of the Chapter reference is made to the judgments in a number of cases

The legal consequences in Great Britain of the contraction of leptospiral diseases depend on the circumstances in which and the place where the disease was contracted This, in each case, must be a question of fact decided by evidence In deciding this question a Court or Tribunal upon whom the decision rests will attach great importance to the expert opinions of medical specialists with experience in diagnosing and treating such diseases

If a person contracts such a disease in his own house or in circumstances where no blame can be placed upon another person then clearly no liability can be attached to anyone If however, such disease is contracted by a person upon the premises of another then different considerations will arise, as this Chapter will seek to show

The most important manner of contraction to consider is that which takes place 'by reason of the nature of his employment' (to use the words of the old Workmen's Compensation Acts) Under the Workmen's Compensation Act 1925, certain diseases, set out in a schedule to the Act, were held to entitle the sufferer to weekly payments (or a lump sum to his dependants in the case of death) where 'the disease is due to the nature of any employment in which the workman was employed at any time within the twelve months previous to the date of the disablement' Leptospiral diseases were not included in the original schedule The schedule was added to from time to time and it was not until 1940 that by the Workmen's Compensation (Industrial Diseases) Order 1940 (S R & O 1940 No 221), 'Infection by Leptospira icterohaemorrhagiae' was added under rather special conditions to which it is not now necessary to refer as the Workmen's Compensation Acts ceased to apply to cases arising after 5th July 1948

The liability for making such payments in respect of diseases contracted by reason of the nature of the employment, and arising after 5th July 1948, has been taken over by the Ministry of National Insurance, and is part of the benefit derived from the payment made by the employer and the employee in respect of the weekly insurance stamp required to be affixed to the

employee's card By section 55 of the National Insurance (Industrial Injuries) Act 1946, such an Insured Person is insured against any prescribed disease which is due to the nature of his occupation If he contracts such disease he, or his dependants if he dies, are entitled to the payments or benefits laid down by the Act By the National Insurance (Industrial Injuries) (Prescribed Diseases) Regulations 1948 (S I 1948 No 1371) the diseases and the nature of the occupations are listed and the Insured Person is entitled to benefit if he contracts such disease by reason of such occupation This list includes ' infection by Leptospira icterohaemorrhagiae ' and the nature of the occupation is set out as ' work in rat-infested places' ' For removal of doubt ' the disease is deemed, unless the contrary is proved, to be due to the employment, if the insured person was employed in the listed occupation on the day he developed the disease or within a month of that day

The Acts of Parliament and the Statutory Regulation and Instrument referred to above are the only ones in which direct reference is made to leptospiral infections My attention has been drawn to the Rat Prevention and Destruction Regulations of 1942 made in Australia (p 214) These Regulations lay down procedures which have to be adopted in regard to the cutting of sugar cane and the precautions to be taken by those engaged in such occupation The object of these Regulations is to minimise, so far as possible, the contraction of leptospiral diseases Clearly similar Regulations would never be made in Great Britain since sugar cane is not grown here, but there is power at any time to make Regulations setting out steps which must be taken to minimise the risk of the contraction of leptospiral diseases in any industry or industries *e g*, fish gutting, where there is a known risk of such contraction

Leptospiral infections and the destruction of rats are nowhere mentioned in the Factories Acts, though these Acts cover a multitude of risks incidental to work in factories It is relevant to notice that by section 66 of the Factories Act 1937, medical practitioners attending to or called in to visit a patient whom they believe to be suffering from any one of a number of diseases contracted in a factory are obliged to notify the Chief Inspector of Factories of any case of any of the diseases referred to in

that Section or the Orders subsequently made adding further diseases to the list of those which are notifiable The word 'factory' has a very wide definition and would include *e g* a fish gutting establishment or a place where food processing was carried on Leptospiral infection is not one of the diseases of which notification must be given in England, but at any time by Order made by the Minister of Labour, leptospiral infection can be added to the list of diseases which medical practitioners have to notify In the same way the Agriculture (Safety, Health and Welfare Provisions) Act 1956 gives to the Minister of Agriculture power to make Regulations compelling employers of agricultural workers to keep records of the occurrence of particular diseases amongst their employees and to give notification of the contraction of such diseases It may be that when further Regulations come to be made, leptospiral diseases will be one of the diseases which have to be recorded and notified

The obligations of an employer to his employees is not confined to the carrying out by the employer of the provisions of any Acts of Parliament or Statutory Regulations relating to the employment The Common Law imposes upon him duties which have been defined by the Courts on countless occasions In 1891 Lord Herschell then sitting in the House of Lords said 'It is quite clear that the contract between employer and employed involves on the part of the former the duty of taking reasonable care to provide proper appliances, and to maintain them in a proper condition and so to carry on his operations as not to subject those employed by him to unnecessary risk'[1] Though that definition was laid down by him as long ago as 1891 it is still an accurate summary of the law to day and has been expressly repeated and approved on many recent occasions in the House of Lords and other Courts

The difficulty of applying the Common Law to any particular case varies from case to case and each must depend upon its particular facts The first fact to which the Court would attach the greatest importance is the degree of risk in the particular employment Thus an employer of persons working in sewers would undoubtedly be held to have a much higher obligation upon him than an employer of persons whose employment was

[1] *Smith* v *Baker & Sons* (1891 A C 325 at p 362)

unlikely to bring them into places where rats were found This degree of liability was described in the words of Lord Macmillan in the House of Lords in 1946 as follows 'The sound view, in my opinion, is that the law in all cases exacts a degree of care commensurate with the risk created'[1]

The next fact the Court would consider would be the steps which the employer had taken to minimise that risk, always considering the degree of risk referred to above In a case where the risk was considerable the Court would hold that considerable steps must be taken to minimise the risk so far as practicable In cases of claims in respect of leptospiral infection the first question to be considered would necessarily be what steps had the employer taken to reduce and keep down, so far as possible, the rat population in the place of employment Thereafter the tests which I have found applied in cases concerning sewer workers who have contracted leptospiral diseases, or their dependants in death cases agree with those set out in the paragraph 'Hygiene of Human Individuals' of Chapter XIV I would only add that in my opinion a Court would further require an employer to give full warning to his employee of the nature of the risk involved and instruction in the precautionary measures to be taken and to see that such measures are carried out This last requirement was set out by Lord Justice Denning 'He (the employer) must set in force a proper system by which they (the employees) use the appliances and take the necessary precautions, and he must see that they adhere to it He must remember that men doing a routine task are often heedless of their own safety and may become slack about taking precautions'[2]

In the event of a particular employer falling short of the standard of care required in the circumstances of any particular case he would be held liable to pay damages to his employee or to the dependants if death had resulted When it is remembered that such damages awarded at the present time to the widow and dependants of a deceased man frequently amount to sums in the region of £4,000 the financial consequences of a failure to fulfil the standard required may be considerable Such sum is awarded without regard to any payment or benefit which the

[1] *Read* v *J Lyons & Co Ltd* (1947 A C 156 at p 173)
[2] *Clifford* v *Charles H Challen & Son Ltd* (1951 1 K.B 495 at p 497)

widow has received under the National Insurance (Industrial Injuries) Act 1946 But in the case where death has not occurred one half of the value of such payment or benefit for a maximum of five years is taken into account in assessing any damages due for loss of earnings or profit in respect of the claim [1] The importance of this last provision is seen when it is realized that the contraction of a leptospiral disease at work may result in two claims by the sufferer; one against the National Insurance Fund and one against his employer

This liability may well fall upon an employer who was ignorant of the risk to his employees of working in places where disease carrying animals existed The Common Law does not necessarily accept ignorance as an excuse, and adopts the test of what a 'reasonable man' should have known This test was defined by Lord Macmillan in the House of Lords

'Legal liability is limited to those consequences of our acts which a reasonable man of ordinary intelligence and experience so acting would have in contemplation The standard of foresight of the reasonable man is, in one sense, an impersonal test It eliminates the personal equation and is independent of the idiosyncrasies of the particular person whose conduct is in question Some persons are by nature unduly timorous and imagine every path beset with lions Others, of more robust temperament, fail to foresee or nonchalantly disregard even the most obvious dangers The reasonable man is presumed to be free both from over apprehension and from over-confidence, but there is a sense in which the standard of care of the reasonable man involves in its application a subjective element. It is still left to the Judge to decide what, in the circumstances of the particular case, the reasonable man would have had in contemplation, and what accordingly the party sought to be made liable ought to have foreseen ' [2]

There are further legal circumstances under which a person who contracts a leptospiral infection may have a valid claim against another The Common Law has for a long time recognized that those who go upon the land or premises occupied by another upon the invitation or with the permission of that other have a duty owed to them The duty owed to such persons was defined as long ago as 1866 by Mr Justice

[1] Law Reform (Personal Injuries) Act 1948
[2] *Glasgow Corporation* v *Muir* (1943 A C 448 at p 457)

Willes 'He [the person invited] is according to an undoubted course of authority and practice entitled to the exercise of reasonable care by the occupier to prevent damage from unusual danger of which the occupier knows or ought to know [1] That definition is certainly good law to day and has been frequently approved by the Courts since 1866 Nowadays the definition can properly be said to define the duty owed by the occupiers of land or premises to all those coming on their land or premises other than trespassers What then would be the legal consequences if for example a person were to go to an open air swimming bath artificially constructed or on the banks of a river and in the banks or surroundings of which rats lived and by reason of that fact that person contracted a leptospiral infection while upon those premises? The danger would certainly be an unusual one in the legal sense namely that it was unexpected Did the occupier know or ought he to have known? This must be a question of fact in each case to be decided on the evidence available If he knew of the presence of rats he would probably still be liable since the association of rats with disease in general is common knowledge It is only when the Courts are considering the question of whether or not an occupier who did not know of the unusual danger ought to have known that difficult questions arise The standard laid down is that of the reasonable man referred to earlier, but the complete answer to this question if complete answer there can be is more suitable to legal text books than the present work One thing is quite clear and that is that persons who are the occupiers of land or premises where rats live may well find themselves legally liable to anyone except trespassers who contract leptospiral disease as a result of coming into contact with the necessary organism upon land or premises unless such occupier has taken reasonable steps to prevent such danger In the case of the unusual danger of contracting leptospiral diseases the reasonable steps required would undoubtedly include the destruction of rats The liability is one to pay such person or his dependants if he is dead damages which may well be considerable

On 6th June 1957 the Occupiers Liability Act received the

[1] *Indermaur v Dames* (1866 L R 1 C P 274 at p 287)

Royal Assent and will come into force on 1st January 1958 This Act lays down a new duty on occupiers of premises towards visitors on their premises, 'The common duty of care' It further states that this new duty shall be 'in place of the rules of Common Law', which I have outlined above so far as I consider they are relevant to the legal questions with which this Chapter deals However, in my view, the broad legal principles upon which liability may well be held to rest on occupiers of premises (including vessels, vehicles and aircraft) in respect of leptospiral infections contracted on their premises will be very similar to those which I have already indicated

The legal consequences of the contraction of leptospiral diseases lie entirely in the realm of civil law No breach of Criminal Law is involved if through the negligence or otherwise of one person, another person contracts such a disease There is one Act which does deal with rats and their destruction, though leptospiral diseases are nowhere mentioned The Rats and Mice (Destruction) Act was passed in 1919 No doubt one of the main objects in view when the Act was passed was to prevent, or at least minimise, the spreading and carrying of diseases by rats and mice The Act lays down that 'Any person who shall fail to take such steps as may from time to time be necessary and reasonably practicable for the destruction of rats and mice on or in any land of which he is the occupier, or for preventing such land from becoming infested with rats and mice' shall be liable to a fine not exceeding five pounds The Act also gives local authorities power to serve notices on occupiers (including masters of ships) to take steps to destroy the rats and mice (and in the case of masters of ships to prevent their escape) If the occupier fails to do so after the notice is served on him then he is liable to a fine not exceeding twenty pounds and the local authority can enter on his land or premises, carry out the destruction themselves and recover from the occupier the cost of so doing The Act has certainly been used on many occasions to serve notices on occupiers to get rid of rats and mice, and steps are taken by the local authority to see that their notices are complied with, including in appropriate cases prosecutions for failure to comply

The legal position therefore remains that the presence of rats on property occupied by an individual whether that property is

a house, a factory, land or a ship may result in his having a notice served on him under the above Act and his possible conviction and fine for a breach of the Act The consequences in Civil Law of the presence of rats may mean that a third party contracts leptospiral disease and the occupier, as the person responsible in law on the basis set out in this Chapter, may well find himself liable to pay that third party or his dependants, if he is dead, damages in money, which damages may well be heavy, and if the contraction took place to an insured person working in a rat-infested place the Ministry of National Insurance will have to make money payments

CHAPTER XVI

PIGS : CATTLE : SHEEP : GOATS : HORSES

PIGS

L pomona

L pomona was isolated from pigs slaughtered in Australia (Johnson, 1939, 1950, Wellington, Stevenson and Ferris, 1951) and from pigs in Batavia by Mochtar (1940) and Collier (1948 b) Widespread infection of pigs has been detected in Switzerland (Gsell and Rimpau, 1944 a) and in the U S A (Bohl and Ferguson, 1952) The infection of pigs has been found in several other countries in Europe and in other continents, as in Italy (Babudieri, 1949 b), France (Kolochine-Erber and Collombier, 1950), Germany (Mitscherlich quoted by Gsell, 1952) and New Zealand (Kirschner *et al*, 1952) In Argentina the serotype was discovered in pigs by Savino and Rennella (1944) who first named it *L suis* but later (1949 a) identified it as *L pomona*

Experimental infection of pigs has been studied in many countries Schmid and Giovanella (1947) infected a pig by rubbing a culture of *L pomona* over its snout The animal showed no signs of illness, but leptospires appeared in the urine on the fourteenth day When they infected 6 other pigs, 3 developed a moderate degree of fever with conjunctivitis and lack of appetite, 1 showed considerable weakness of the hind limbs and another had neck stiffness for a considerable time

Burnstein and Baker (1954) studied pigs killed at various times up to 20 weeks after inoculation with *L pomona* Of 7 pigs killed during the febrile period, no gross changes were found in 4, the other 3 showed scattered petechial haemorrhages in the lungs, and the livers of 2 were pale, slightly mottled and friable In animals killed 3 or more weeks after infection the only lesions noted were in the kidneys which showed white spots of varied shapes and sizes on the exterior. As they developed, these white areas appeared as depressions

extending into the medulla, and the capsule overlying them was difficult to strip. After longer intervals the kidneys showed scarring, became shrunken, and bands of fibrous tissue were seen on the surface and extending into the parenchyma.

When 4 pregnant sows were infected experimentally by Ryley and Simmons (1954), 3 aborted 3 to 4 weeks before full term, and the other farrowed a week before the expected time. Of the 37 piglets which they produced, 34 were either born dead or died a few minutes after birth. A number of the foetuses were decomposed when expelled, indicating that they had died several days before birth, and leptospires were not isolated in cultures from them. Many of the piglets that died at or just after birth showed focal necrosis of the liver, and leptospires were isolated from 12.

Ferguson and Powers (1956) infected sows by the conjunctival and intranasal routes at different stages of pregnancy. They found that infection in the first and third months seemed to have little effect on reproduction, while none of the three gilts exposed during the second month produced a normal litter. Infection produced intravaginally immediately after mating did not disturb pregnancy.

The epizootology of *L. pomona* infection of pigs was extensively studied by Gsell (1952) and his collaborators in Switzerland. They found that the serum of 59 per cent of 193 pigs slaughtered in various parts of Switzerland agglutinated *L. pomona* to 1/100 or higher, and 27 per cent to higher than 1/800. It appears that chronic leptospiruria is a more common sequel of porcine than bovine, ovine, equine or human infection by *L. pomona* (Borg Petersen and Fennestad, 1956 b). The danger of conjunctival infection by the splashing of urine into the eyes of farm animals, and of intranasal infection by sniffing and grouting is emphasized by several workers.

Schmid and Giovanella (1947) showed that young pigs were infected by association with an older pig which was excreting leptospires in the urine, and the young ones showed leptospiruria after 12 days' contact. Leptospires have been recovered from piglets born of experimentally infected sows as mentioned above, and in the U.S.A., Bryan, Rhoades and Willigan (1953) found them also in the tissues of aborted foetuses. In addition, infection of 4 out of 4 pigs by contact with experimentally

infected calves was demonstrated by Morter and Morse (1956) In Denmark, Borg-Petersen and Fennestad (1956 b) found *L pomona* in 3 out of 14 striped field mice (*Apodemus agrarius*) which occur in Denmark only on the islands of Lolland and Falster The serum of 5 out of 153 pigs from these islands agglutinated *L pomona* significantly whereas pig sera from elsewhere in the country were negative They believed therefore that the striped field mouse was the principal carrier of *L pomona* in Denmark

TABLE XXVIII

SYMPTOMS ATTRIBUTED TO LEPTOSPIROSIS IN 54 HERDS OF PIGS 1952 54
(after Bryan 1955 a)

	Per Cent
Abortion	85
Reduced milk	7
Fever	22
Haemoglobinuria	11
Anaemia	9
Jaund ce	13

In many countries pigs appear to suffer little clinical disturbance from infection, but evidence is accumulating that this is not always the case Gochenour, Johnston, Yager and Gochenour (1952) believed that infection of suckling pigs resulted in general unthriftiness and lowered resistance to other diseases, such as hog cholera Abortion and infected foetuses were found in the U S A by Bryan *et al* (1953) Bryan (1955 a) found in Illinois that infection of pigs was present in three-quarters of the Counties of the State, in 28 per cent of the herds that were examined and in 18 5 per cent of the pigs tested The percentage of herds showing symptoms attributable to infection are given in Table XXVIII High rates of abortion in infected herds were also recorded by Bohl, Powers and Ferguson (1954) and Powers, Bohl and Ferguson (1956)

L hyos
(Syn *L mitis* Johnson)

L hyos has been found to be the cause of mild or inapparent infection of pigs in a similar way to *L. pomona* and often in

the same countries, but it is not so widespread as *L pomona* in the areas affected Its presence was described in pigs in Australia by Johnson (1942, 1950) and Wellington *et al* (1951), in Switzerland by Gsell and Wiesmann (1948), in Argentina by Savino and Rennella (1944) and in the Belgian Congo by van Riel and van Riel (1954)

L icterohaemorrhagiae

Small outbreaks or single cases of infection of pigs due to *L icterohaemorrhagiae*, characterized by jaundice and a fairly high mortality, were reported from the Netherlands (Klarenbeek and Winsser, 1937), England (Field and Sellers, 1951) Scotland (Nisbet, 1951), Ireland (Power, 1951), the U S A (Bohl and Ferguson, 1952) and Denmark (Fennestad, 1956) A heavily endemic state of infection of pigs by this serotype was found in Western Samoa by Johnson (1943), and Collier (1948 b) found serological evidence of infection in Batavia

Affected animals show signs of inappetence and fever for a day or two before jaundice develops, and a few days later they become prostrate and die soon afterwards All the organs are jaundiced, and numerous petechial haemorrhages may be found in the lung, intestinal walls, and retroperitoneal tissue Microscopically, many of the liver cells are necrotic, in the kidneys, the tubules are extensively damaged, but most of the glomeruli only show thickening of Bowman's capsule

L canicola

L canicola was isolated from the urine of pigs during an investigation into human cases of canicola fever in the U S A (Williams *et al*, 1953) Seiler *et al* (1956), in Scotland, found significant agglutinins for *L canicola* in sera from 46 out of 76 pigs in a piggery where human cases of canicola fever had occurred They believed it likely that the infection had reached the piggery from a dog (which was not traced) and then passed from pig to pig They stated that no marked symptoms of disease were observed in the pigs but that leptospires were seen in the urine

An epidemic in Israel among young pigs about four months

old was reported by van der Hoeden (1956) The animals showed fever, anorexia, listlessness, weakness and convulsions These animals were also infected with *Salmonella cholerae-suis* as were the young pigs infected by *L pomona* recorded by Gochenour, Johnston *et al* (1952) van der Hoeden killed one of the pigs and examined the kidneys He found petechial haemorrhages in the cortex, chronic interstitial nephritis, degeneration of the epithelial cells of the tubules, and infiltration of the glomeruli The part played by the leptospires in these outbreaks is difficult to assess, on account of the double infection

OTHER SEROTYPES

Serological evidence has been recorded of infection of pigs by *L autumnalis* and *L pyrogenes* in Batavia (Collier, 1948 b) by *L bataviae*, *L grippotyphosa* *L poi* and *L sejroe* in Denmark (Fennestad, 1956), and by *L ballum* in Czechoslovakia (Kmety *et al*, 1956)

CATTLE

L grippotyphosa

Nikolajev (1946) stated that *L grippotyphosa* was the cause of epizootics in cattle in the U S S R, and at about the same time the disease was intensively investigated in Israel where it affects mainly adult cows When the causative organisms were first isolated in Israel Bernkopf (1946) thought they represented a new serotype for which the name *L bovis* was suggested by Btesh (1947) Later it was agreed that *L bovis* is a synonym of *L grippotyphosa*

Clinically, Freund (1947) recognized three forms of the disease One is a *peracute* form which occurs during pregnancy or when the animals are suffering from another acute infection In this form the onset is sudden with high fever, the urine becomes black, and the mucous membranes are deeply jaundiced, general stasis of the digestive system is pronounced, the urine contains albumin, haemoglobin, and bile pigments in large amounts Blood urea is high in the terminal stages, and death usually ensues in three to seven days Gayot (1955) who

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amounts Blood urea is high in the terminal stages, and death usually ensues in three to seven days Gayot (1955) who

studied the condition in Tunisia, noted the occurrence of cutaneous ulcers in acute cases. The lesions exuded a sticky fluid which coagulated on the hair, producing hard plaques. As the ulcer healed the plaques loosened and finally dropped off leaving bare patches of skin.

Postmortem examinations of cattle which had died after *experimental infections* were made by Ungar and Bernkopf (1947). The liver was of normal size and consistency, and the kidneys appeared normal apart from an icteric tinge and some hyperaemia of the cortex. Microscopically, scattered foci of central necrosis were observed in the livers of some but not all animals, and small groups of liver cells were enlarged and showed discrete vacuolation of the cytoplasm. Macrophages containing haemosiderin were seen in the periportal tissue in several cases. There were cellular infiltrations round the smaller bile ducts in which there were often bile thrombi containing desquamated epithelial cells. In the kidneys the glomeruli were moderately hyperaemic. In many areas the tubules were distended; they contained hyaline or cellular casts, and a number of the epithelial cells were necrotic. Focal accumulations of round cells were constantly present, particularly around the convoluted tubules. Small numbers of leptospires could be seen in sections of both liver and kidney stained by silver impregnation.

Many cases among milking cows are of a *subacute* form, lasting about two weeks, from which the majority recover. The onset is slow, and the first signs are usually noted in the milk which becomes pinkish and contains blood clots. The milk flow is reduced, sometimes to a few drops of viscid fluid. Jaundice appears, and is accompanied by cessation of rumination and stasis of the digestive system. The kidneys are greatly enlarged, and return to normal size only slowly during convalescence, which is prolonged for two months or more.

In the *chronic* and *recurrent* forms the symptoms are less pronounced; in some outbreaks neither jaundice nor haematuria occur, and abortions may be the *only indication of infection*. In Israel, van der Hoeden (1953 b) observed differences in se[illegible] which may have been related to the bree[illegible] of cattle. *In one herd of Fresian* cross bre[illegible] 19 out of 95 became seriously ill and

10 died; 30 others showed slight symptoms of disease The rest of the cows remained clinically healthy though all but 7 developed antibodies in the blood. van der Hoeden (1953 c) also examined sera of a second herd of 131 indigenous Arab cattle during the period when an enzootic (referred to below) occurred among goats None of the cattle developed clinical signs of infection, but over 50 per cent showed positive agglutination titres to *L grippotyphosa*

L pomona

Strains of leptospires, later identified as *L pomona* by Gochenour, Yager and Wetmore (1950), were isolated from cattle in the U S A by Baker and Little (1948) and by Little, Beck and McCahon (1950) Reinhard (1953 a) estimated that probably tens of thousands of new cases occur annually, and he gathered evidence of leptospirosis (mostly due to *L pomona*) among cattle in 28 States of the U S A Many, possibly even the majority of these infections, are either inapparent or produce only mild, fleeting signs of disease However, this type of infection acts as a frequent cause of abortion in pregnant cows, and the animals may continue to excrete leptospires in the urine for weeks or months, and so spread infection throughout the herd Morse (1955) stated that leptospirosis is the fourth most important disease of cattle in the U S A , and that it is probably responsible for an annual loss of 100 million dollars to the farming community York (1951) found widespread leptospirosis in New York State and recorded that in infected herds 50 to 70 per cent of animals were affected Of those affected, 5 per cent died, 5 to 10 per cent were severely ill, and up to 25 per cent of pregnant cows aborted

In the State of Illinois, Bryan (1955 a) found infection in 77 out of 92 of the Counties, and in 31 per cent of 2,056 herds of cattle, or 16 per cent of the 26,000 animals tested Among 125 of the infected herds disturbances were attributed to leptospirosis as shown in Table XXIX As a rule, the animals showed symptoms resembling those of the subacute form of the disease produced by the Palestine strains of *L grippotyphosa* with fever, haematuria, and blood stained milk, but some animals were severely affected and died as the result of the

attack In similar outbreaks in New Zealand, Kirschner *et al* (1952) noted that milking cows which survive may suffer from damage to the udder with consequent cessation of lactation

In young animals, *L pomona* causes 'redwater of calves', an acute febrile illness with a high mortality, characterized by jaundice and haemoglobinuria The disease has been described *in Australia by* Sutherland, Simmons and Kenny (1949) and Wellington *et al* (1951) *and in New Zealand by Kirschner et al* (1952) Salisbury (1954) considered that in New Zealand

TABLE XXIX

SYMPTOMS ATTRIBUTED TO LEPTOSPIROSIS IN 125 HERDS OF CATTLE, 1952-54

(after Bryan, *1955* a)

	Per Cent
Abortion	58
Reduced milk	47
Fever	38
Haemoglobinuria	30
Anaemia	19
Jaundice	15

it is an important cause of abortion in cows and of red-water in calves In the outbreak observed by Sutherland *et al*, all the calves which died were less than 4 months old The onset of the disease was sudden, with fever, the urine was dark red *in colour, and the mucosae were* jaundiced. Death occurred from a few hours to one day after the symptoms were first noted At autopsy widespread jaundice was present, the *kidneys were congested and showed a few subcapsular petechial* haemorrhages Calves which recovered took six to eight weeks to regain normal health

Experimental infection of calves, adult cattle and pregnant cows was studied in the U S A by Reinhard and Hadlow (1954), Morter and Morse (1956), Morse and McNutt (1956) and Ringen and Bracken (1956), and in Denmark by Fennestad and Borg-Petersen (1956) Subcutaneous injection of *L pomona* (or other serotypes) into cows caused fever, mild or severe illness and occasionally death or abortion of a foetus Morter and Morse showed that contact infection passed to pregnant heifers, pigs and a goat Ringen and Bracken infected cattle with *L pomona* by instillation into the conjunctival sac, or by

nebulization in the nostril, or by holding a shaved foot in diluted infected urine for a few minutes

Borg Petersen and Fennestad (1956 b) found that, in Denmark, a striped field mouse (*Apodemus agrarius*) which occurs only on the islands of Lolland and Falster, is the chief carrier of *L. pomona* in that country On those islands 13 out of 200 cattle were found by serological tests to be infected but the infection did not occur elsewhere in the country

L. canicola

From Israel van der Hoeden (1955 a & b) described epidemics and sporadic cases of leptospirosis caused by *L. canicola* The infection caused a fever of short duration in cows, with lack of appetite weakness jaundice haemoglobinuria and decreased milk yield Calves were much more seriously affected, and in some outbreaks nearly half the animals died or had to be slaughtered van der Hoeden considered on epidemiological evidence that jackals not dogs constituted the primary focus of infection in 3 out of 4 outbreaks The sera of 6 out of 7 jackals showed significant agglutinin titres for *L. canicola*, and strains isolated from the kidneys of two were proved to belong to that serotype

Other Serotypes

Infections of young calves with *L. icterohaemorrhagiae* have been recorded by Maria and Quevedo (1947) in Argentina, by Field and Sellers (1950) in England by Mantovani (1953) in Italy by Baxter and Pearson (1956) in Ireland and by Markov and Rybkina (1957) in the U S S R Jaundice and haematuria were the outstanding features, and the mortality was high An enzootic among cattle, characterized by haemoglobinuria and with a mortality of about 30 per cent occurred in Japan during 1949 The sera of 24 cattle which had recovered from the disease were tested by Yamamoto (1951) Leptospiral antibodies were present in every case, but three different serotypes appeared to be involved, namely *L. australis A*, *L. autumnalis* and *L. hebdomadis*

In Central Africa, van Riel and van Riel (1955) found serological evidence of infection of 42 out of 124 cattle (35 per

cent), the leptospires concerned were *L australis A*, *L bataviae*, *L grippotyphosa*, *L pomona*, *L hebdomadis*, Icterohaemorrhagiae group and the Butembo type Savino and Rennella (1945-8) diagnosed serologically infection with *L hyos* in 20 per cent of a group of cattle in Argentina, and Johnson (1950) found cattle in Australia infected by the same serotype van Riel and Bienfet (1953) found that the serum of 10 per cent of 278 cattle in Belgium gave evidence of infection by one or other of four serotypes or serogroups Borg Petersen and Fennestad (1956 a) estimated from serological tests that 7 to 8 per cent of Danish cattle over one year of age had leptospirosis due to the sejroe-saxkoebing or poi-javanica groups, to *L grippotyphosa*, *L pomona*, *L bataviae*, *L icterohaemorrhagiae* or *L ballum* Gsell (1952) recorded serological evidence in Switzerland of 23 per cent infection of 612 cattle with one or other of seven serotypes

UNIDENTIFIED INFECTIONS

Outbreaks and sporadic cases of leptospirosis in which the strains isolated were not identified, or in which the diagnosis was made by the observation of leptospires in sections of tissues obtained postmortem were described by Jungherr (1944), Marsh (1945), Mathews (1946), Sutherland and Morrill (1948), Reinhard, Tierney and Roberts (1950) and Zaharija (1955) Nefed'ev (1949) studied extensive epidemics of leptospirosis in cattle in the U S S R in 1948

SHEEP

Sheep appear to be relatively resistant to leptospirosis, though Melanidi, Tzortzaki and Debonera (1933) were able to infect them experimentally with *L icterohaemorrhagiae* Wirth (1937 a) recorded that in Austria sheep are known to have been infected, and van der Hoeden (1953 c) found serological evidence of infection with *L grippotyphosa* in Israel He stated however that in the few clinical cases seen, the disease ran an atypical course and ended in early recovery. Nefed'ev (1949) recorded infection of sheep in various parts of the U S S R in 1948 The majority of cases occurred in summer

An outbreak in New Zealand investigated by Hartley (1952)

was of a much more serious nature, and caused the death of 12 lambs and 2 ewes The only symptom noted was haematuria Autopsies carried out on 2 lambs showed widespread jaundice, the livers were yellowish-brown in colour, and the kidneys had a brownish appearance, more marked in the cortex Histological examination revealed extensive centrilobular necrosis of the liver together with bile ductule proliferation The collecting tubules of the kidneys contained much coarse, granular, orange-red pigment In the epithelial cells of some of the proximal convuluted tubules there was globular eosinophilic material, and in others a brownish yellow pigment Leptospires were seen in sections of liver and kidney stained by Levaditi's method No bacteriological investigation was made of the affected animals However, the following year during another outbreak in which the clinical and pathological findings were essentially the same, strains were isolated by guineapig inoculation The strains were not identified, but sera from recovered cases had high agglutinin titres for *L pomona*

Beamer, Hardenbrook and Morrill (1953) described ovine leptospirosis in the U S A, on a farm where there had probably been cases among calves some six months earlier Young pregnant ewes were chiefly affected and invariably aborted A day or two after abortion, the ewes became sickly, lost condition, developed haematuria and jaundice, and 15 out of 19 died At autopsy the livers appeared fatty and the kidneys were swollen Leptospires were isolated from one of the foetuses, and the sera of some of the affected ewes contained leptospiral antibodies The authors did not however state which serotype was responsible for the epizootic

In Germany, Mochmann (1955) found serological evidence of infection of 1 out of 220 sheep by *L canicola*, and in Central Africa van Riel and van Riel (1956) found that the serum of 3 out of 72 sheep showed significant agglutination titres with *L bataviae*, leptospires of the Icterohaemorrhagiae group and the Butembo type respectively

GOATS

Goats were known to be susceptible to experimental infection with *L icterohaemorrhagiae* (Ghetti, 1922; Wirth 1937 a) but

no instances of spontaneous leptospirosis had been recognized until van der Hoeden (1953 c) investigated cases of ictero haemoglobinuria among goats in Israel The presence of agglutinins for *L grippotyphosa* in the sera of sick goats first suggested that the disease might be of leptospiral origin In a later outbreak leptospires were demonstrated in stained sections of tissues from fatal cases, and final confirmation was obtained when a strain of *L grippotyphosa* was isolated from a goat at autopsy immediately after death

The symptoms comprised inappetence, icterus and the passage of red or dark brown urine No definite febrile reactions were noted, though the death rate was high, amounting to more than 40 per cent of the affected animals in one instance Many pregnant female goats aborted

At autopsy the tissues were deeply jaundiced The kidneys showed venous congestion with degeneration and haemorrhages in the medula There were swelling, degeneration and desquamation of the epithelial cells of the tubules, and hyaline and cellular casts were often present There was some evidence of interstitial nephritis with mononuclear infiltration, especially of the perivascular and periglomerular regions The glomeruli were swollen, their capillaries empty, and there was some proliferation of both visceral and parietal layers of Bowman's capsule The liver showed venous congestion and degeneration of liver cells, most marked around the central veins Slight infiltration of mononuclear cells was present in the portal spaces, especially around the bile ducts Many leptospires were seen in sections of liver and kidney stained by Levaditi's method

van Riel and van Riel (1956) found that the serum of 12 out of 353 goats (3 4 per cent) in the Belgian Congo and Ruanda-Urandi agglutinated one or other of five serotypes to a titre of at least 1/100 The serotypes were *L bataviae*, leptospires of the Icterohaemorrhagiae, Grippotyphosa or Hebdomadis serogroups and the Butembo type

HORSES AND OTHER EQUIDAE

Comparatively little disease of horses has been clearly proved to be due to leptospires, although in many countries serological

evidence has been found of subclinical infection by various serotypes present in the regions concerned For instance, Kathe (1943) reported that the serum of 35 out of 162 healthy horses in Germany agglutinated *L grippotyphosa* to a titre of 1/1,000 Wagener and Mitscherlich (personal communication to Gsell, 1952) found that the serum of 15 out of 109 healthy horses in Hanover agglutinated leptospires—*L grippotyphosa* 8, *L pomona* 3, *L canicola* and *L icterohaemorrhagiae* 2 each van der Hoeden (1953 c) found serological evidence in Israel of infection by *L grippotyphosa* in 4 out of 5 horses and 6 out of 12 donkeys This confirmed similar findings in horses, mules and donkeys in the same country by Jacusiel *et al* (1948) In East Germany, Mochmann (1955) found that the serum of 25 out of 1,065 horses agglutinated *L canicola* to a titre of at least 1/400 Fennestad (1956) reported serological evidence of infection of horses by *L icterohaemorrhagiae*, *L poi*, *L grippotyphosa*, *L sejroe* and *L saxkoebing* in Denmark, and Salminen (1956) found leptospiral antibodies in the sera of a high proportion of the horses which he tested in Finland

In England, Alston and Broom (unpublished data) tested the serum of 108 healthy horses and found agglutinins to *L ictero haemorrhagiae* to an end titre of 1/100 in 20 per cent, 1/300 in 54 per cent, 1/1,000 in 17 per cent and 1/3,000 in 2 per cent, results with *L canicola* were of the same order Similar findings in various countries are recorded below in an outline of investigations of periodic ophthalmia

Lubashenko and Novikova (1947 a & b) have studied equine leptospirosis in different parts of the U S S R From 1940–46 they saw 111 spontaneous infections with a mortality of 66 per cent on farms where leptospirosis was present in other animals, such as cattle and silver foxes The sick horses had a high temperature for two or three days, then developed jaundice, and this was followed by petechiae on mucous membranes, bald spots on the skin, haemolytic anaemia, and haemoglobinuria in the terminal stages The illness lasted for 5 to 18 days, and peracute, subacute, chronic and atypical forms were seen

Strains of leptospires were isolated by guineapig inoculation and by culture from 2 horses, but the diagnosis usually depended on the results of agglutination tests A titre of 1/400 was the minimum accepted as proof of infection, but titres up to

1/40,000 were sometimes obtained [The serotypes concerned are not shown in the available translations of these papers]

In Yugoslavia, Zaharija (1953 b) observed 8 horses infected by *L pomona*, confirmed by serological tests and isolation of leptospires The horses were ill for 5 to 7 days with fever, jaundice and conjunctivitis, and leptospiruria was found late in convalescence towards the thirtieth day There were no deaths

Roberts, York and Robinson (1952) isolated *L pomona* from 2 out of 6 horses which had a short illness with symptoms of fever, dullness, anorexia and, occasionally, jaundice These animals all lived on the same farm in the U S A , and were ill at the same time One mare was delivered of a premature foal which died deeply jaundiced two days after birth Another mare had marked photophobia, lachrymation and greying of the cornea There seemed to be coagulation of the aqueous humour, and severe periodic ophthalmia was diagnosed The mare's general condition improved with dihydrostreptomycin therapy, but the severe affection of the eyes did not improve until aureomycin was given intravenously for three days, when complete resolution of the ophthalmia occurred and there was no recrudescence This mare foaled during her treatment An agglutination test was first made 24 days after the onset of illness, and the serum agglutinated *L pomona* to a titre of 1/512 None of the other 5 horses developed periodic ophthalmia This outbreak is interesting in showing that leptospiral infection of horses can produce symptoms including jaundice, disturbance of pregnancy and ophthalmia

There is evidence that horses excrete leptospires in the urine after experimental infection with *L pomona* (Yager, 1953) and in this way may transmit infection to other animals or to man It may be presumed that they become infected from rodents, dogs, pigs, cattle or other farm animals

PERIODIC OPHTHALMIA (RECURRENT IRIDOCYCLITIS)

Controversy exists about how great a part—if any—leptospiral infection plays in periodic ophthalmia Crawford (1954) outlined the knowledge of the epidemiology of this condition and the numerous theories of its causation The disease has been known since the fourth century and has been named periodic

ophthalmia, recurrent ophthalmia, iridocyclitis, uveitis and moonblindness Crawford considered 'recurrent iridocyclitis' a very suitable name Diagnostic features are (i) the manner in which acute attacks clear up, only to recur after intervals of one month to a year or longer and (ii) the sudden onset of the acute attacks At present it is most commonly found in low swampy areas in Central Europe and in the U S A east of the Rocky Mountain watershed It was common in Great Britain before 1880 in large coaching stables and stables of tramway horses especially in low-lying districts At present it is uncommon and sporadic in Britain

Infection by bacteria (including *Brucella suis*), filterable viruses, or nematodes as well as avitaminosis and allergy have been suggested as aetiological agents, but no single cause has yet been confirmed for all clinical cases

A detailed account of the clinical forms of the disease was given by Crowhurst (1954) He stated that the disease is very rare in foals and is most frequent in animals from 1 to 8 years old Prognosis is very grave in the vast majority of cases Blindness will occur sooner or later, and in almost 50 per cent of cases the second eye becomes involved also Treatment with atropine reduces the risk of synechiae but does not arrest the disease In Crowhurst's opinion neither riboflavin nor various antibiotics nor antihistamines have been effective The use of cortisone has yet to be tested adequately

Jones (1942) described the morbid anatomy and histology in detail, and concluded that the disease was primarily a recurring fibrinolymphocytic iridocyclitis which gradually progressed to a chronic degeneration of all the intra ocular structures Ashton (1954) concluded that the microscopical picture of the disease in the horse differed little from many cases of recurrent iridocyclitis in man, where the aetiology is equally obscure He considered that the histopathology was consistent with an allergic, leptospiral or viral origin, but was not in harmony with vitamin or protein deficiency or with invasion by pyogenic organisms or helminthic larvae.

In 1948 Heusser, Gsell, Kanter and Wiesmann presented serological evidence that periodic ophthalmia of horses is a leptospiral disease The evidence they put forward was gathered from replies to a questionnaire sent to Swiss veterinary surgeons

(Gsell, 1952) The disease was reported in 276 horses and appeared to be associated with cold wet seasons and damp marshy regions Leptospiral agglutination tests were done with sera from 263 of these 276 horses, from 91 horses with other forms of eye disease, and from 291 healthy horses The results showed that among the 263 horses with periodic ophthalmia the sera of 78 per cent agglutinated leptospires at a dilution of 1/400 or higher, and only 6 per cent were completely negative By contrast, among 382 healthy horses and horses with other eye diseases the sera of only 14 per cent agglutinated at 1/400 or higher, and 61 per cent were negative Further evidence was obtained from a horse in which serum agglutination was negative 13 days after the onset of the illness in November 1944, was positive to 1/8,000 in January 1945, fell to 1/2,000 in March, rose with recurrence of inflammation to 1/4,000 on two dates in April and May, and to 1/8,000 in June Two years later the reaction was almost negative, but was found to be positive again at 1/3,000 in March 1948

Agglutination titres in the sera of affected horses were highest with *L grippotyphosa* in 118 out of 206 (58 per cent) In the other cases the serotypes involved appeared to be *L pomona*, *L australis*, *L sejroe* and *L icterohaemorrhagiae* The sera of healthy horses and of horses with other eye diseases which contained agglutinins, also reacted most frequently with *L grippotyphosa* The presence of agglutinins in 39 per cent of healthy horses or in those suffering from other eye diseases was taken to mean that many horses may be infected by leptospires and that ophthalmia develops in a proportion of these animals as almost the only clinical manifestation

The Swiss workers could not demonstrate leptospires in the aqueous humour, but they considered that their failure was due to lack of early cases Gsell recorded that Kathe successfully cultured leptospires from the aqueous humour of one horse, and Heusser (1952) produced iridocyclitis in two foals by extra-ocular injection of *L pomona*

Kathe informed Gsell (*loc cit*) that in Mecklenberg, Germany, he examined 6 horses with moonblindness and confirmed its leptospiral origin serologically He considered 3 cases to be due to *L icterohaemorrhagiae*, and the others to *L grippotyphosa* Rimpau (1947) found in Munich that the serum of 19 out of 20

horses with moonblindness agglutinated *L grippotyphosa*, in 11 cases to titres of 1/1,000 to 1/16,000, in 6 cases between 1/200 and 1/600 and in 2 to 1/100 Rossi and Kolochine-Erber (1954) reported a higher percentage of reactions in the sera of 288 horses with iridocyclitis than in the sera of 87 stable mates when they were tested against nine serotypes, Zaharija (1952 a & b, 1953 b) found in Yugoslavia leptospiral antibodies (serotypes not mentioned) in 50 to 70 per cent of all horses which he tested, and in 6 out of 6 suffering from ophthalmia

In the U S A, Yager, Gochenour and Wetmore (1950) investigated periodic ophthalmia in 35 horses, and found that the serum of 34 agglutinated *L pomona* in dilutions of 1/100 or higher (in fact 24 of the sera agglutinated up to at least 1/10,000) By contrast, the sera of only 13 out of 86 healthy horses agglutinated *L pomona* to 1/100 or more, and only 3 to 1/10,000 or more Also in the U S A, Woods and Davis (1950) found agglutinins for *L icterohaemorrhagiae* up to 1/5,000 in 12 out of 12 horses with active ophthalmia, and up to 1/100 or 1/1,000 in 5 out of 5 with chronic ophthalmia By contrast, the sera of only 4 out of 30 healthy horses agglutinated that serotype to 1/1,000 or more, 8 were positive to 1/100, 14 to 1/10, and 4 were completely negative.

The ophthalmia in a mare which was 1 of the 6 horses apparently infected by *L pomona* on a farm in the U S A has already been mentioned (p 250) Strains were isolated in culture from two of these animals, but culture was not attempted from the mare with ophthalmia, her serum taken on the twenty-fourth day agglutinated *L pomona* to a titre of 1/512 Bryans (1955) continued the study of these 6 horses, and observed periodic ophthalmia in 2 of them 1 year and 2 years after the illness of the whole group He also found antibodies for *L pomona*, *L icterohaemorrhagiae* or *L canicola* in 30 per cent of 512 brood mares, but none in 492 weanling horses Serum of 20 out of 23 horses with ophthalmitis, or a history of

ophthalmia was not proved.

In England we have investigated (unpublished) 9 cases of periodic ophthalmia in horses, and after bacteriological, sero-

logical and histological examination have not found convincing evidence of a leptospiral cause Six of the horses were suffering from a primary acute attack in one or both eyes, and the others had chronic ophthalmia of long standing The sera of these 9 horses agglutinated *L icterohaemorrhagiae* and *L canicola* to titres of 1/100 to 1/1,000 but, as recorded above, we found that 93 per cent of 108 healthy horses also had agglutinins for *L icterohaemorrhagiae* in dilutions of 1/100 or higher The question needs more study in many countries for it must be admitted that the frequency of iridocyclitis in human leptospirosis makes a theory of leptospiral ophthalmia in horses seem attractive

CHAPTER XVII

DOGS : FOXES : JACKALS : CATS : BATS : PRIMATES

DOGS

Leptospirosis was considered by Krumbein and Frieling (1916) to be the probable cause of an attack of jaundice in a certain dog because two men in close contact with it developed Weil's disease Courmont and Durand (1917) showed that fatal jaundice could be regularly produced in young dogs by injection of guineapig liver containing leptospires They also infected dogs percutaneously through shaved or epilated skin, and through skin which was intact after the hair had been clipped Uhlenhuth and Fromme (1919) identified leptospiral infection in a dog by finding leptospires in smears of kidney and liver of a guineapig inoculated with the dog's tissues

Lukes (1924) saw spirochaetes in the kidneys of 7 out of 8 dogs which had died with symptoms of haemorrhagic gastroenteritis and ulcerative stomatitis He named the organism *Spirochaeta melanogenes canis* and successfully transmitted the infection to guineapigs Since the organism was lost it is not possible to say to which serotype it belonged

Okell, Dalling and Pugh (1925) proved that 'yellows' was caused by *L icterohaemorrhagiae*, but Klarenbeek (1927) recognized two clinical forms in both of which leptospires were found in the kidney The icteric form was the same as the disease investigated by Okell *et al* but signs of nephritis predominated in the other form (Stuttgart disease) Klarenbeek considered that Stuttgart disease was probably leptospiral in origin, and this was proved in 1931 when Klarenbeek and Schüffner (1933) isolated from the urine of a dog in Utrecht a strain of leptospires which differed serologically from other known serotypes The new strain was called Roesel (Hond Utrecht IV) Later, the Roesel strain was named *L canicola*

and its low degree of pathogenicity for guineapigs was established (Schuffner, 1934)

There has been a gradual clarification of the clinical effects of *L icterohaemorrhagiae* and *L canicola* in dogs The opinion is accepted (Klarenbeek, 1938, Wirth, 1937 b, 1939, Mills, 1948) that in general *L icterohaemorrhagiae* causes jaundice and sometimes severe nephritis with uraemia, in a small minority of cases nephritis occurs without jaundice *L canicola*, on the other hand, causes jaundice uncommonly For example, Klarenbeek (1938) found that jaundice was present in 47 out of 57 (80 per cent) canine illnesses due to *L icterohaemorrhagiae*, in contrast with 3 out of 94 (3 per cent) due to *L canicola* during the five years 1933–38 in the Netherlands As an exception, Meyer, Stewart-Anderson and Eddie (1939 b) emphasized its occurrence in California In Klarenbeek's series, death occurred in 50 per cent of those infected by *L icterohaemorrhagiae* and 40 per cent infected by *L canicola* Nephritis with uraemia occurs in about half the cases The uraemic form of infection, with haemorrhages and often severe gastro-enteritis has been known in the past as dog typhus, ulcerative stomatitis, haemorrhagic gastro enteritis or Stuttgart disease. All or nearly all these cases are due to *L canicola* Infection with either serotype may cause mild symptoms or be inapparent

Both serotypes have been recorded from almost all parts of Europe, Asia, North and South America and Australasia Table XXX gives the results of some representative surveys of infection by *L icterohaemorrhagiae* and *L canicola* in dogs in various countries, listed according to the dates of the published records The figures represent either isolation of leptospires or serological tests It will be noted that on the whole *L canicola* is responsible for a higher proportion of infections than *L icterohaemorrhagiae*

L icterohaemorrhagiae

EPIZOOTOLOGY —Sporadic cases occur among house and farm dogs, but epizootics are more common when numbers of dogs are housed and fed together in conditions which favour infestation by rats, as was emphasized by Okell *et al* and by

Stuart (1946 a). Infection is mainly caused by contact with rat urine, but the disease can be transmitted from dog to dog—and also from dog to man.

A seasonal incidence of infection has been noted in several countries, as in the Netherlands where Klarenbeek (1938) found that 29 per cent of 57 overt infections occurred in the months of September, October and November of the years 1933–37. The reason for this was not clear, and he surmised

TABLE XXX

SELECTED SURVEYS OF INFECTION RATES OF DOGS WITH *L. icterohaemorrhagiae* OR *L. canicola*

Year	*Author*	*Country*	*Per Cent Positive*	
			L. ictero-haemorrhagiae	L. canicola
1936	Uhlenhuth & Zimmermann	Germany	6	12
1937	Borg Petersen & Jacobsen	Denmark	35	2
1939 b	Meyer *et al.*	U.S.A.	1.5	14
1939	van der Walle	Belgium	15	30
1940	Beuvery Asman	Netherlands	13	26
1940	Snapper *et al.*	China	—	10
1940	Babudieri & Castagnoli	Italy	33	3
1941	Greene	U.S.A.	—	29
1941	Raven	U.S.A.	8	25
1942	Alston (unpublished)	England	—	34
1943	Fraga de Azevedo	Portugal	5	13
1943	Alicata & Breaks	Honolulu	20	19
1944	Savino & Rennella	Argentina	6	25
1946 a	Stuart	Scotland	9	40
1948	Broom & MacIntyre	England	2	21
1949 c	Guida	Brazil	13	18
1951 b	Broom	Eire	—	30
1953 a	Zaharija	Yugoslavia	7	45
1955	Mochmann	Germany	11	26

that a similar variation in the degree of leptospiruria of rats might be responsible.

SYMPTOMS AND COURSE OF ILLNESS.—Okell *et al.* investigated eight dogs and tissues from another, and from three of the animals they isolated leptospires which were virulent for guinea-pigs. It is possible that some of the other dogs were suffering from infection by *L. canicola*, which had not been recognized at that time. With this reservation, their clinical and pathological

descriptions are still very valuable They divided the clinical course into two patterns which they named the hyperacute or haemorrhagic, and the icteric types Later Joshua (1949) recognized a subacute type

Hyperacute Type—The onset is sudden, with high temperature (104°–106°F , 40°–41°C), intense depression, shivering and tenderness of muscles of the neck or abdomen Haemorrhagic herpes of the lips, bleeding from the gums and other mucous membranes and vomiting are frequent Urine is scanty, may be bile-stained, and contains albumin Enlargement of lymph glands, petechiae or conjunctivitis may occur Icterus is not usually present, and death commonly supervenes in a few hours to a few days after the onset

Icteric Type—The illness begins variably In the rapidly fatal form with jaundice the onset is acute, while in others the disease may be so insidious that the dog is not thought to be sick until jaundice is evident The temperature is raised at first, but falls to normal or below as jaundice appears The animal tends to hide away and be inactive, but not to be so depressed as in the hyperacute type Bronchial râles may be heard, and epistaxis occurs uncommonly The gums are yellow but the mouth is otherwise healthy Vomiting is a fairly constant feature and blood may be present in the vomit Constipation is usual, faeces clay like and sometimes blood-streaked Intussusception of the small intestine can often be diagnosed by palpation and is often the immediate cause of death The urine is coloured with bile and contains albumin Conjunctivitis frequently occurs Acute cases may die within two days of the onset while milder cases may survive a week or longer Conjunctivitis or keratitis may be a sequel to recovery

Subacute Type—The symptoms are generally similar to those of subacute infection by *L canicola* (p 261) Slight icterus may develop after four to six days and the dog seems very ill without serious symptoms Response to treatment is poor, and if recovery occurs convalescence is slow

MORBID ANATOMY AND HISTOLOGY—At autopsy, jaundice is general except in hyperacute cases and haemorrhages of varying sizes may be found in almost any tissue of the body Intussusception of the intestine and prolapse of the rectum are frequent

The kidneys may be congested, or pale and jaundiced The liver may be enlarged and congested or pale and icteric In sections of the liver Monlux (1948) noted disruption of the cord structure The kidneys in his series showed many petechial haemorrhages in the cortex, which was usually ischaemic, and also in the medulla which tended to be hyperaemic The proximal convoluted tubules showed marked degeneration, similar to the changes seen in mercury poisoning

L canicola

EPIZOOTOLOGY —Infection of dogs by *L canicola* has been detected by culture of urine, of kidney or other tissues or by serological tests The percentage of dogs in which *L canicola* has been detected varies widely from place to place according to whether healthy dogs sick dogs in general or dogs suspected of leptospirosis were tested but it appears that in some regions from 30 to 40 per cent of apparently healthy dogs may show antibodies indicative of either former active infection or of a symptomless carrier state (Table XXX) All breeds of dogs are liable to infection (Meyer *et al* 1939 b) but Joshua (1949) believes that low-to ground breeds, like Scottish terriers are especially often at risk

Sex Incidence and Source of Infection —It is generally but not quite unanimously believed that male dogs are oftener infected than female This was the experience for example of Raven (1941) in Pennsylvania of van der Walle (1939) in Belgium, of Klarenbeek and Winsser (1938) in the Netherlands and of Meyer *et al* (1939 b) in California where the ratio of male to female was 67 18 in fatal infections On the other hand, Beuvery-Asman (1940) in Belgium, and Joshua (1949) in London recorded almost equal incidence in the sexes In a general review of the literature, Rosenberg (1951) estimated that dogs have been reported 3 to 5 times as frequently as bitches

The difference in sex incidence may be related to the habit, which is specially prevalent among males, of smelling and licking the genitals and excreted urine of other dogs which may be carriers Dogs may continue to excrete leptospires in the urine for weeks or months after recovery from infection. Indeed McIntyre and Montgomery (1952) demonstrated the

organisms in sections of kidneys taken from dogs four years after clinical recovery from naturally acquired infections with *L canicola* In addition, Joshua suggested that after sexual maturity in the male (at about 8 to 10 months) urination may be the cause of contact between the preputial hairs and the wall or lamp-post against which other dogs have urinated Joshua believes that low-to-ground breeds, which like the Scottish terrier have a long whisker of hair round the genitals in both sexes, may be infected by direct contact with infective urine on the ground Infection of dogs of either sex by swimming in contaminated water is established (Winsser, 1943, Joshua, 1949) Dogs and bitches may infect one another by coitus but it appears that infection from contaminated food or drink is uncommon

Age Incidence—The evidence about the commonest age at which infection of dogs occurs, and the relative susceptibilities of young and older dogs is not conclusive Statements are found frequently in the literature to the general effect that infection is commoner in older animals, but published reports do not fully support that view Most of the information is derived from serological tests of healthy dogs which have recovered from earlier infections For example, Raven (1941) recorded the ages of 50 dogs (in and around Philadelphia) which showed serological evidence of past infection The infection rate was 19 per cent for dogs up to 1 year of age, 25 per cent for those of 1 to 3 years and 46 per cent for 3 to 10 years This indicates that nearly one-fifth of the dogs had become infected before they were 1 year old On the other hand, Meyer *et al* (1939 b) stated that the disease was rare in California under the age of 1 year However, natural infection of puppies has been found at or soon after birth (Roos *et al*, 1937, Senthille *et al*, 1945) Puppies were infected experimentally by Walch-Sorgdrager (1939) and by Klarenbeek and Winsser (1938) and the latter observers noted that 10 out of 11 of the young dogs in their series developed jaundice Since the risk varies from place to place and time to time it would seem that young dogs are clearly susceptible, and there is at any rate no evidence that the first year of life is a resistant age Restriction of general contact with other dogs may be a protective element for part of the first year

Seasonal and Regional Incidence — Klarenbeek (1938) compared the seasonal incidence of infection of dogs by *L icterohaemorrhagiae* and *L canicola* Of the former 29 out of 57 infections (50 per cent) occurred in September, October and November but only 35 out of 94 (35 per cent) of the latter were in these three months The other 39 cases were distributed irregularly throughout the rest of the year Others like Joshua (1949) found most infections by *L canicola* between late October and April inclusive *i e* the wetter periods of the year, but our own figures for 319 infections do not show a seasonal trend Variation in the numbers of acute infections from year to year in the same areas has often been observed and has been attributed, in part, to changes in wet or dry conditions of the ground

Symptoms and Course of Illness —The clinical features of the disease have been described by many observers, including Wirth (1939) Meyer *et al* (1939 b) Lovell (1943) and Joshua (1949) The following account is taken mainly from Joshua s publications

Acute canicola fever is the syndrome known to clinicians as Stuttgart disease, dog typhus haemorrhagic gastro enteritis or ulcerative stomatitis It comprises acute vomiting, rapid dehydration and collapse occasionally the passage of blood-stained faeces and, if the dog survives long enough, rapid necrosis and sloughing of the buccal mucosa and tongue The mortality rate before the use of penicillin was high, death occurring in 30 hours to 4 days Some writers including Meyer *et al* (1939 b) have described jaundice in acute infections

[illegible] ossible to [illegible] The sy[illegible] amage in many organs occasionally occurs Icterus may be present and the temperature may be raised early in the disease Symptoms commonly include profound depression, vomiting muscular pain in the early stages, and a tucked up appearance usually associated with pain in the renal area Thirst may be in abeyance or excessive with corresponding oliguria or polyuria Episcleral injection is very characteristic when it occurs Ulceration of the buccal mucosa is common and the

tongue is dry and often shows a brick-red or brownish discolouration Marked *enlargement of the kidneys* may occur, and it is often possible to palpate the left kidney very easily Albumin may be present in the urine in slight degree or be absent, and traces of bile are common In the majority of cases death, when it occurs, is due to uraemia Rimpau and Kalich (1948) noted that signs of meningeal irritation occur in dogs and that muscular weakness may sometimes be found after recovery

It is generally agreed that infection by *L canicola* is one of the important causes of subacute or chronic nephritis in dogs

TABLE XXXI

BLOOD UREA CONCENTRATION AND PROGNOSIS

(after McIntyre and Stuart 1949)

Stage and Type	*Blood urea in mg per 100 ml*	*Prognosis*
Primary renal		
(1) Severe	200–350	Very grave
	> *350*	*Usually hopeless*
(2) Mild	40–200	Good
Secondary renal		
(1) Severe	> 150	Grave
(2) Mild	40–150	Serious

McIntyre and Stuart (1949) found serological evidence of this type of leptospirosis in *90* per cent of *130* dogs which *had* detectable renal disease, but in only 23 per cent of 140 other dogs The serological diagnosis was confirmed in a number of cases by the isolation of the organism from blood culture This is one of the highest rates of correlation between infection by *L canicola* and renal disease in dogs McIntyre and Stuart divided their renal cases into primary (acute) and secondary (subacute), and found that the blood urea concentration was a useful index in prognosis (Table XXXI) In the chronic form the disease is usually progressive and has a fatal termination

The appearance of severe renal symptoms may be delayed for months or years after recovery from the initial illness, and for that reason Weipers (1949) considered that primary, secondary and tertiary leptospirosis are suitable terms for the possible course of the disease

MORBID ANATOMY AND HISTOLOGY—Renal lesions are predominant in all stages, and they were fully described by McIntyre and Montgomery (1952), who also reviewed the results of previous workers These authors examined the kidneys of 21 dogs which had been destroyed during the acute stage, and of 13 destroyed up to four years after recovery from an acute attack of canicola fever

In the acute group the kidneys showed, characteristically, a broad band of yellowish or yellowish-white colour situated at the boundary of the cortex and medulla Microscopically, this was seen to be due to intense nodular infiltration of the boundary zone with non-granular leucocytes which surrounded but did not involve the glomeruli This may indicate the presence of a renal shunt mechanism such as that described by Trueta *et al* (p 92) Cortical tubules were separated by many non-granular cells The tubular epithelium showed cloudy swelling, and leptospires were usually abundant within the convoluted tubules The nodular lesions were relatively avascular, but the adjacent *vasa recta* were dilated and congested

All the dogs of the chronic group had pale contracted kidneys with fine granular surfaces, and sclerosis of the renal artery and its branches was common Microscopically, the main change was focal and diffuse fibrosis, much of it perivascular (Fig 14) Diffuse interstitial fibrosis of the cortex also was not uncommon, and there was focal deposition of connective tissue at the boundary zone representing residual scars of lesions seen in the acute stage Glomerular damage was slight, and took the form of thickening of Bowman's capsule with some damage to the tufts and some adhesions of tuft to capsule In general the changes were those of slowly mounting hypertension Leptospires were relatively scanty and occurred in groups or agglutinated clumps, sometimes in hyaline material They were demonstrated at all periods up to four years—which was the longest included

OTHER SEROTYPES

L medanensis (Kouwenaar and Wolff, 1929, 1930), *L bataviae* and *L pomona* (Mochtar and Collier, 1939) and *L wolffii* (Schuffner *et al*, 1939) were all isolated from dogs in Indonesia. Serologically, infection by *L schuffneri* has been detected in Indonesia (Collier and Mochtar, 1939 a), by *L pomona* in Italy (Babudieri and Castagnoli, 1940) and in Germany (Brede, 1951), by *L pyrogenes* in Germany (Brede, 1951), by *L sejroe* and *L saxkoebing* in Denmark (Fennestad, 1956)

FOXES

Acute infection by *L icterohaemorrhagiae* has been found in wild foxes (Dunkin and Laidlaw, 1925, Uhlenhuth and Fromme, 1930) Smith (personal communication) investigated an outbreak of the infection in a silver fox farm in Scotland when three animals died Catchpole (1934) made a similar report on clinical evidence alone Lubashenko and Novikova (1947 a) referred to infection of silver foxes on farms where cattle and horses were also infected

JACKALS

van der Hoeden (1955 a) found infection among jackals in Israel Serum of 8 out of 10 possessed significant agglutinins for *L canicola* and the serotype was isolated from four Epidemiological evidence showed that in the same area jackals provided a reservoir from which cattle were infected At necropsy the kidney of one jackal showed many small yellowish white nodules in the cortex and at the cortico-medullary junction, and microscopical examination revealed small haemorrhages, multiple foci of interstitial infiltration, and large clumps of leptospires in many of the convoluted tubules

CATS

Leptospiral infection of cats is uncommon and very rarely causes recognizable disease Esseveld and Collier (1938 a) in

Java isolated 13 strains of leptospires from the kidneys of 500 cats and one strain from the blood of a cat Of these strains 8 were *L bataviae* and 6 *L javanica* The cats which were found to be infected were all 1 5 kg or more in weight Similarly, among older cats the serum of 25 to 45 per cent agglutinated *L bataviae*, and the serum of 30 per cent agglutinated *L javanica* Esseveld and Collier did not find any disease attributable to these infections and considered that the cats were infected from rats

Surveys elsewhere have shown even less evidence of infection of cats van den Brekel (1938) thought, on rather slender evidence, that a cat was the source of a human case of Weil's disease In Denmark, Tennestad (1956) recorded serological evidence of infection by *L icterohaemorrhagiae*, *L poi*, *L saxkoebing* and *L bataviae* Hemsley (1956) in England found agglutinins for *L canicola* in the sera of three cats suffering from chronic nephritis The histological changes in the kidneys were very varied and there appeared to be no relationship between the clinical picture and the severity of the lesions Changes were most marked in the glomeruli, some of which were destroyed and replaced by fibrous tissue

In a similar survey in London Joshua and Broom (unpublished data) tested serum from 200 cats and found low agglutination titres for *L icterohaemorrhagiae* or *L canicola* in a proportion of the specimens They failed however to isolate leptospires from the few cases of acute jaundice which they were able to investigate

In Germany, Otten Henze and Goethe (1954) tested serum from 236 cats for complement-fixing antibodies to four or more serotypes, and only one serum gave a weak reaction with *L canicola* and *L sejroe* By agglutination tests, 1 out of 86 showed a titre of 1/100 with *L icterohaemorrhagiae*, and another with *L canicola* and *L sejroe* Experimental infection of cats with *L icterohaemorrhagiae* or *L canicola* produced a low titre of antibodies and only slight disease

BATS

In Indonesia, bats act as carriers of *L schüffneri* (p 135) and also of *L cynopteri* which has not so far been known to cause

disease in man or other animals (Collier and Mochtar, 1939 a & b)

PRIMATES

An epidemic of an acute and rapidly fatal disease among chimpanzees in a zoo in French Guinea was described by Wilbert and Delorme (1927) Of the affected animals 23 out of 24 died, and Wilbert himself contracted the infection while carrying out a postmortem examination on one of the animals Strains of leptospires were isolated from this chimpanzee and also from the man The same authors (1928) concluded that they were *L icterohaemorrhagiae*

In a survey carried out by Erber (1932) agglutinins for *L icterohaemorrhagiae* were detected in the serum of one gorilla, 1 out of 9 *Macacus cynomolgus* and 33 out of 279 *Cynocephalus papio* or *C hamadryas* Some of these monkeys *had been in captivity in France for a considerable time before* the tests were made, but others were bled less than a week after their arrival from West Africa

CHAPTER XVIII

TREATMENT AND CONTROL OF LEPTOSPIROSIS IN DOMESTIC ANIMALS

TREATMENT

The treatment of leptospiral disease in farm animals has not yet been clearly established by controlled observations, but some of the reports of the use of antibiotic drugs or antiserum in the treatment of naturally occurring and experimental infection will be mentioned The study of treatment of experimental leptospirosis in laboratory animals is summarized in Chapter XIII Until more evidence has been collected on the treatment of farm animals it may be suspected that, as in man antibiotic or serum treatment which does not begin until a few days after the commencement of the illness may not be effective *Carefully controlled experiments are needed and until these have been made the following dosage of antibiotic drugs per lb body-weight given intramuscularly each day for five days may be suggested* penicillin 1,000 units, oxytetracycline (terramycin) 3 mg or tetracycline hydrochloride 5 mg

If renal failure develops, treatment following along the lines advocated in human leptospirosis (Chapter XIII) might be useful We have seen no reports of its use in farm animals, but somewhat similar régimes evolved for the treatment of both acute and chronic nephritis in dogs are described later

Immune Serum

In dogs infected with *L icterohaemorrhagiae* Okell *et al* (1925) considered that antileptospiral serum proved of value in treatment, but the method never appears to have been widely adopted Specific immune serum has also been used in the treatment of leptospiral disease of cattle and horses in the U S S R The serum was produced by hyperimmunization of animals of these two species with two serotypes, and was used

for treatment in a single dose of 100 ml subcutaneously, or in two such doses with an interval of three days between them (Lubashenko and Novikova, 1947 b, Lubashenko, 1949) [The translated versions of these reports do not record the degree of control, but it was stated that the treatment was successful]

ANTIBIOTIC DRUGS

PIGS —Nisbet (1951) treated 20 pigs with sodium penicillin as soon as a tentative diagnosis of leptospirosis was made, 12 pigs had previously died on the same farm and *L ictero haemorrhagiae* had been isolated from 2 of them The drug was given in a dose of 100,000 units intramuscularly on each of three successive days, only one pig died, and it was jaundiced before treatment began Two pigs on another farm recovered after similar treatment although they were jaundiced before treatment began Serological tests on several of the recovered animals did not show a significant titre of antibodies

In pigs infected with *L pomona* Ferguson, Lococo, Smith and Hamdy (1956) found that sufficient chlortetracycline (aureomycin) added to the ration to provide each sow with 1 g per day did not eradicate the kidney carrier state in 15 gilts, although the amount of abortion and of neonatal mortality was reduced On the other hand, Howarth (1956) believed that chlortetracycline given as 200 mg per lb of food for fourteen days, or 3 mg of oxytetracycline per lb weight of the animal intramuscularly daily for five days, did eradicate the leptospires from the kidneys Baker, Gallian, Price and White (1957) successfully eliminated the urinary excretion of *L pomona* in experimentally infected pigs by feeding a ration containing 500 g and 1,000 g of oxytetracycline per ton for fourteen days

CATTLE —Bryan (1955 b) concluded that no therapeutic effect was obtained when cattle naturally infected with *L pomona* were treated with penicillin, streptomycin, chlortetracycline, or a combination of oxytetracycline and streptomycin He based his conclusions on the fact that agglutinin titres were not reduced by the treatment, and made no attempt to determine whether the animals were excreting leptospires either before or after treatment On the other hand, Ringen, Bracken, Kenzy and Gillespie (1955) eliminated the carrier state in cattle

experimentally infected with *L pomona* by giving dihydrostreptomycin intramuscularly in doses of 5 mg per lb bodyweight 12-hourly for three days Similarly Ringen and Bracken (1956) claimed that they abolished leptospiruria due to experimental infection with *L pomona* by tetracycline hydrochloride in dosage of 2 5 or 5 mg per lb body weight given once daily for five days

DOGS —Brunner and Meyer (1949) and Joshua (1950) reported disappointing results with penicillin therapy in spontaneous infections with *L icterohaemorrhagiae*, but the former workers concluded that streptomycin was effective in doses of 40 mg per kg body weight daily for three or four days

Joshua and Freak (1947) treated six dogs suffering from *L canicola* infection by means of penicillin. In four treated for 4 to 8 days within a few days of the beginning of the illness, there was very rapid improvement and all recovered, in a fifth the illness had lasted six weeks before penicillin was given and recovery was accompanied by signs of permanent kidney damage The sixth dog had probably been ill for four weeks, and it died after nine days treatment with signs of recent acute pneumonia as well as leptospirosis Brunner and Meyer (1949) discovered that penicillin did not eradicate leptospires from the kidneys, and that the dogs developed leptospiruria after the cure of the acute illness A subsequent course of streptomycin (40 mg per kg of body-weight, daily for four or five days) was needed to eliminate leptospires completely

The importance of supportive treatment was emphasized by Witter (1950) He recommended the administration of fluids in the form of 5 per cent glucose in normal saline, Ringer's solution gelatine, and protein hydrolysates to counteract dehydration One or two blood transfusions in addition to these fluids will improve the results Frequent enemeta of 5 per cent sodium carbonate solution remove irritant materials from the intestine and reduce intestinal ulceration Injectable crude liver and thiamine hydrochloride offset anaemia and improve the appetite During convalescence, a high protein diet with iron and arsenic tonics will hasten recovery

Martin, Knight and Pittaway (personal communication) have obtained dramatic improvement in cases showing acute nephritis by intravenous transfusion of glucose-saline (see below) and

the administration of 500 mg vitamin C together with antibiotics They consider however that the dog s diet should contain no meat for six to nine months after recovery

A method of treating the chronic nephritis which frequently follows recovery from *L canicola* infections has been elaborated by the same workers They regard the blood urea level as a most important index of the dog s condition and consider a concentration of 60 mg per 100 ml or more as evidence of uraemia The average value in dogs suffering from chronic nephritis is from 100 mg upwards in severe cases it may be between 200 and 300 mg

Treatment is begun with a transfusion of 500 ml of normal saline containing 5 per cent glucose but less is given if the animal develops rapid breathing panting and excessive trembling during the administration For support a treatment the animal
receives 500
vitamin B_1
consist of milk puddings milk foods and glucose in water and milk together with 10 to 20 mg vitamin B_1 daily by mouth.

Blood urea estimations are made at intervals of 2 or 3 days and further transfusions given If the concentration was originally from 100 to 120 mg per 100 ml, it should be reduced to about 50 mg by five transfusions and can be maintained at that level by the meat free diet with the addition of vitamin B_1 This diet must be continued for the rest of the dog's life

The dogs should be examined at intervals of three to six months when the original response was satisfactory about 80 per cent can be expected to live for many years In some cases 0 25 to 0 5 mg digoxin twice daily by mouth is needed if heart disturbances poor circulation and venous congestion occur

CONTROL

Elimination of Carriers

This would obviously be the ideal method of control but it could seldom be attempted for practical and economic reasons It was however the means adopted by Jones *et al* (1945) to prevent the spread of canine leptospirosis at a War Dog Reception Centre in Virginia, U S A Serum for agglutination tests was taken from each dog as soon as possible after it

reached the Centre and the animal was quarantined for at least two weeks The dog was destroyed if leptospiral antibodies were present in the serum or if symptoms of leptospirosis developed during quarantine These drastic measures were carried out in time of war, but the decision may have been influenced by the fact that only 1 3 per cent of the 4 368 dogs examined had to be destroyed When the dogs are household pets it might be possible to eliminate the carrier state by giving the course of streptomycin therapy described in the section on Treatment

PREVENTION OF TRANSMISSION

Methods for destroying rodents and for preventing them from gaining access to buildings and contaminating food and water supplies are outlined in Chapter XIV As regards the spread of infection within a herd Coghlan *et al* (1957) noted that in some of the piggeries they investigated the pigs were crowded into pens to maintain a warm temperature and that the pens were hosed daily and were almost always damp These conditions might favour the survival and dissemination of leptospires and they suggested that the pens should be treated with a hypochlorite solution after they had been washed (p 45)

In an outbreak of *L pomona* infection in cattle described by Baker and Little (1948) the disease was thought to spread directly from cow to cow by inhalation The cattle continued to excrete leptospires for about two months after recovery and if the animal urinated from a standing position on to a concrete floor, a spray of droplets was produced which could be inhaled by nearby cattle In such cases the affected animals should be isolated from the rest of the herd until they cease to be carriers

The following measures for the control of leptospirosis among livestock in the U S A were tentatively recommended by Little and Baker (1953)

1 Report outbreaks of leptospirosis to State or Federal authorities

2 Confirm all reported outbreaks of leptospirosis by the employment of appropriate laboratory methods

3 Isolate and quarantine the laboratory confirmed clinical cases Require strict sanitary precautions to minimize the risk of spread of infection to other animals and to man Discard milk from affected lactating animals

4 Obtain immediately and examine serum specimens from all animals on the premises

5 During the outbreak, require the daily recording of temperatures of all animals Segregate those animals with temperatures above 103°F [39 5°C] Isolate and quarantine if leptospirosis is confirmed on laboratory examination of nonclinically diagnosed cases

6 Examine serums of all animals one month following the onset of the outbreak Isolate and quarantine animals found to have contracted the infection during this period

7 Continue monthly serological examinations of all animals for three months after detection of the last acute infection, before permitting reintroduction into the herd, or other movement of the animals under quarantine

The measures advocated by Reinhard (1953 b) were of a different nature He considered that the primary requirement in the U S A was adequate laboratory facilities for making serological surveys of herds His suggestions were that farmers with serologically negative herds should segregate newly acquired animals for a month and have their sera tested before allowing them to mix with the rest of the stock Farmers with diseased animals should be advised to make no additions to their herds for at least 6 months, and to keep new calves and unaffected stock isolated from the infected herd for 6 to 9 months after the last case had occurred York (1951) had previously expressed similar views and recommended that serological tests should be made before cattle were transported from an infected area to one which was free of the disease

Because of the high carrier rate among pigs, Kirschner *et al* (1952) suggested that these animals should be kept completely separated from cattle Gochenour (1952) pointed out that care must also be taken to ensure that the water supplies of clean herds cannot be contaminated by the urine of infected animals

IMMUNIZATION

PASSIVE —Okell *et al* (1925) considered that the inoculation of dogs with antileptospiral serum was a useful prophylactic measure during outbreaks in kennels of infection with *L icterohaemorrhagiae* Similarly, Lubashenko (1949) claimed that the hyperimmune serum he used in treatment was effective

in prophylaxis in horses and cattle, under both experimental and field conditions

ACTIVE —A vaccine for immunizing dogs against 'yellows' was prepared by Dalling and Okell (1926) from a suspension of the livers of guineapigs infected with *L icterohaemorrhagiae* In small field trials the vaccine appeared to provide a good level of protection Ottosen (1946) inoculated about two thousand dogs with a formolized vaccine of *L canicola* and only two developed leptospirosis during the period of observation—which is not definitely stated but was apparently between six months and one year

The vaccination of farm animals has been studied mainly in the U S S R, the U S A and New Zealand Nefed ev (1949) believed that a vaccine given in two doses was effective in reducing the incidence of disease in cattle in infected herds He stated that the vaccine had been used in tens of thousands of cattle and sheep in the U S S R with good results Lubashenko (1949) claimed that a chinosol vaccine was 100 per cent effective in protecting foxes and calves In the U S A, York and Baker (1953) and Bramel and Scheidy (1956) have studied the prophylactic use of bacterin made from *L pomona* inactivated by formalin or by freezing and thawing Their results in small numbers of cattle and horses were encouraging

Immunization of sheep by a vaccine of *L pomona* was tested experimentally by Webster and Reynolds (1955) in New Zealand Twenty sheep resisted challenge doses given three weeks and eleven months after the administration of the vaccine, while 16 out of 20 unvaccinated control animals showed leptospiruria which lasted up to three months

In spite of these satisfactory reports Morse Allen Pope and Krohn (1955) were sceptical about the value of vaccination in preventing the spread of infection within a herd after an outbreak had begun They pointed out that a diagnosis of leptospirosis was often made only after a number of cows in a herd had aborted If active immunization were begun then a further period of 2 or 3 weeks would elapse before a useful level of protection could be attained By that time from 4 to 6 weeks might have passed since the introduction of the disease into the herd Epizootics due to *L pomona* are often explosive in character, and the whole herd is rapidly exposed to the risk

of infection They considered therefore that the disease would 'probably have claimed most of its victims' before vaccination could confer adequate protection For that reason they decided not to recommend the use of vaccine during an outbreak

In view of these conflicting opinions it is apparent that a true estimate of the value of vaccination in the control of leptospirosis cannot be made until more information is available

LEPTOSPIROSIS IN REGIONS MOST AFFECTED

CHAPTER XIX

REGIONAL OCCURRENCE OF LEPTOSPIROSIS IN THE COUNTRIES MOST AFFECTED*

Japan Germany Indonesia Netherlands France
British Isles Australasia Denmark Switzerland Italy
U S.A South America Israel

In this Chapter an outline is given of the development of knowledge of leptospirosis in man and animals in the countries and regions where the infection has been most studied The first knowledge of the pathogenic leptospires was gained in Japan and much information was obtained soon afterwards in that country and in Indonesia and Malaya European workers took up the study of leptospirosis soon after the publication of the first Japanese work Successful pioneering work has been recorded from Australia since 1933 In North and South America knowledge accumulated slowly until about 1938 and since that time infections of animals and man have been found of increasing frequency In Africa little evidence of the disease has been found except in the Belgian Congo, in some other territories of the west equatorial zone and in Kenya

Table VII gives extensive lists of serotypes found throughout the world In this Chapter only an outline of the recognition of serotypes and some main features of aetiology are given, more detail is contained about the separate serotypes in Chapters VIII–XI

JAPAN

In Chapter I an account is given of the pioneer work in Japan which included a clinical study of Weil s disease, the

* Much of the material contained in this Chapter appears in other Sections but it is considered here with regard to individual regions In addition reference is made to a number of points which are more of local than of general interest and importance

discovery in 1914–16 of the causative organism—*L. ictero haemorrhagiae*—and provided a large proportion of our present knowledge of the epidemiology and serology of the disease Japanese workers discovered two other new leptospiral serotypes in the 'seven-day fevers of Japan' and also found in their *country four serotypes which had first been isolated elsewhere*

In certain country districts in Japan a disease, running a course of about seven days and with symptoms like those of atypical Weil's disease, had long been recognized Opinions differed whether 'seven-day fever' was a distinct entity, but Inada (quoted by Ido *et al*, 1918) who studied the disease in the Fukuoka Prefecture where it is known as 'nanukayami', *concluded that it was an independent affection This conclusion* was confirmed when Ido *et al* (1918) isolated strains of lepto spires from cases of the disease and showed that they differed from *L icterohaemorrhagiae* serologically, and in pathogenicity for guineapigs For the new serotype they proposed the name *Spirochaeta* (=*Leptospira*) *hebdomadis* or in their Japanese publications *S nanukayami* In 1949 Gauld *et al* (1952) found infection by this serotype in sixteen U S soldiers in the island of Okinawa

A similar disease in Shizuoka Prefecture, known locally as 'akiyami', was investigated by Koshina *et al* (1925) Strains of leptospires isolated from patients showed marked differences *in their virulence for guineapigs and were divided tentatively* into two groups, *Akiyami* leptospira A type, and *Akiyami leptospira B type Further investigation proved that the B type* strains were identical with *L hebdomadis*, but that the A type strains represented a new serotype Later Abe (1934) found [illegible] day fever in Nagasaki [illegible] 'hasamiyami', were [illegible] pe and he proposed to name the serotype *L autumnalis* In addition to the common names already mentioned seven day fever is also known in various localities as akke disease, odan *eki*, sakushu *fever, and* sahara fever

Among serotypes previously discovered elsewhere, *L australis A* has been found in Japan where in certain areas, such as along the Tenryu River, it causes a seven-day fever It was for a time named in Japan *L akiyami C, L hebdomadis C* or

L tenryuensis The animal reservoir of this serotype in Japan has not been found

Less commonly L *bataviae* has caused human infections in Japan but the animal reservoir has not been discovered *L pyrogenes* has been found in human patients and in rats, and infection by *L canicola* was first presumed in one patient on immunological evidence, and later found to be the cause of 43 cases of disease in ricefield workers

Infections by *L icterohaemorrhagiae* have been commonest among coal miners, food handlers and some farm workers, the infections by *L canicola* were presumably from dogs, and the other serotypes mostly infected field workers

GERMANY

Weil's article which established a new disease entity was published in 1886 and Goldschmidt (1887) first used the name 'Weil's disease' German writers, such as Jaeger (1892) described other cases of the disease on a clinical basis During World War I, Hubener and Reiter (1915, 1916)—without knowing of the slightly earlier Japanese discoveries—transmitted the infection of Weil's disease in German soldiers to guineapigs, monkeys and rabbits and named the causal organism *Spirochaeta nodosa* Uhlenhuth and Fromme (1915, 1916 a & b) confirmed these findings and named the organism *Spirochaeta icterogenes* These two names are synonyms of *L icterohaemorrhagiae* A monograph 'Die Leptospirose', by Rimpau was published in 1950, and it includes an account of a considerable amount of the German experience on leptospirosis

Infections by *L icterohaemorrhagiae* and *L canicola* have been found in many parts of the country in circumstances similar to those in other lands, but the numbers of such cases are much less than those due to *L grippotyphosa* (Rimpau, 1948, Gaehtgens, 1950, Brede, 1951, Kathe and Engelhardt, 1953) Adam (1950) showed that infections by *L icterohaemorrhagiae* occurred mostly on the coasts of the North Sea and Baltic Sea and in the lower courses of the Rivers Weser and Elbe By contrast field fever (due to *L grippotyphosa*) was found mainly well inland, and canicola fever occurred without special geographical association

Evidence of infection of animals by *L icterohaemorrhagiae* was found in dogs (Uhlenhuth and Zimmermann, 1936, Dahr, 1937), white rats (Roelcke, 1938), wild rats (Fühner, 1950 b), cattle (Kathe, 1943), *Arvicola sherman* (Rimpau, 1943) and, doubtfully, a cat (Otten *et al*, 1954)

Since the end of the last century German clinicians had recognized 'mud fever' as a specific disease occurring among people working in damp fields In 1926 Prausnitz and Lubinski reported that they had cultured leptospires from the blood of patients suffering from the disease, but they failed to subculture the organism or to transmit the infection to animals After Tarassoff (1931) reported his discovery of *L grippotyphosa*, Rimpau *et al* (1938) showed that this organism is the cause of 'mud fever' in the basin of the River Oder, 'harvest fever' in the Danube basin and in the basin of the Elbe, and 'swamp fever' in various parts of the country

The infections by *L grippotyphosa* have been very numerous, for Rimpau (*1948*) found 663 in eight years in South Bavaria, and epidemics of 300 cases among pea harvesters in Lower Saxony, and of at least 300 in pea harvesters and cabbage gatherers in Schleswig-Holstein have been reported (Popp, 1950, Hermannsen, 1954) Litzner and Hahn (1950) and Popp (*1950*) showed that the first of these epidemics was partly dependent on an acute epizootic spread of *L grippotyphosa* in field mice Other groups of cases and isolated infections by the same serotype have been reported from various parts of Germany by Glattkowski (1950), Führer (1953), Naumann (*1950*) and Burggraf (*1950*) Kathe (*1943*) found serological evidence of old or recent infection by *L grippotyphosa* in dogs, horses and cattle

Human infections by *L canicola* have been found frequently in Germany, and Steigner and Messerschmidt (1950) estimated that there may be 3 cases per 100,000 of the population, annually He noticed a seasonal incidence of the disease in man and in dogs, with peaks in late summer and in winter Other collections of cases were recorded by Gunther-Kühne, Rimpau and Schubert (1949), by Rimpau (1948) in South Bavaria, by Hellwich (1950) in Saxony, by Gaehtgens (1950) in Hamburg, and by Brede (1951) in Cologne and district Infection of dogs was studied by Uhlenhuth and Zimmermann

(1936) in Freiburg-im-Breisgau, by Dahr (1937) Steigner (1950), and by Brede (1951) in Cologne and district The last named found 138 dogs infected during 1950 , approximately 80 per cent of these dogs were male

Twelve infections of human beings by *L sejroe* have been found by Rimpau (1948) Infection by this serotype was found serologically in wood mice by Rimpau (1943) and in dogs by Steigner (1950) Rimpau (1948) discovered two human cases of infection by *L australis A*, and Lubbers (1951) suspected infection by *L saxkoebing* in two patients, and demonstrated it in two dogs

L muenchen was isolated from a human infection in Munich (Wolff, 1953 a)

INDONESIA

According to Walch-Sorgdrager (1939) the first recognized case of Weil's disease in Indonesia was reported by van der Scheer (1892) Schuffner (1918) demonstrated leptospires, as well as malaria parasites, in blood films of a patient who died with symptoms of blackwater fever Schuffner proposed the name *icterohamoglobinurica* for these leptospires, but as the strain was not grown in culture its identity is unknown, and the name is not in use

During the next two decades Schuffner and his colleagues made an intensive investigation into fevers of short duration and unknown origin occurring mainly among plantation workers in Sumatra, where leptospires were frequently found in the alkaline natural waters of the island In contrast, the waters of Java are more acid leptospires are almost absent in them and only a few human leptospiral infections were found in that island Leptospires of a wide variety of serological types were isolated *in Sumatra , Collier (1948 a) stated that from human* cases 18 different serotypes had been isolated in culture, and that there was serological evidence for the presence of an additional 3 serotypes In addition 3 more serotypes have been found only in animals These findings are recorded in Table VII In studying the relationships of these strains, the Dutch workers elaborated the serological techniques which are now used as the basis for classifying the genus

NETHERLANDS

Weil's disease was first recognized in the Netherlands in 1924 (Schüffner, 1934) The incidence has been greatest in three provinces below sea level on the West coast, including Amsterdam, Rotterdam, Utrecht and Leyden, where the water is less saline than in northern parts of the Netherlands A seasonal increase in late summer and early autumn was noticed and in Amsterdam, especially, the disease occurred in three groups of people (1) Those whose occupation put them at risk, such as fishermen, bargemen or slaughtermen (2) Those infected by falling into canals by accident or with suicidal intention and who came near to being drowned and were in a helpless state when removed from the water Falling into clean fresh water in lakes did not lead to infection, but only canals which were polluted by human and animal refuse and of which the banks were teeming with rats Infections of this type occurred without seasonal emphasis (3) Infections of bathers and swimmers in canals and in open air swimming baths in which the water was polluted by refuse and infected with *L icterohaemorrhagiae* It is this group that caused the sudden rise in cases from July to October It is probable that the use of the crawl stroke in swimming increases the risk of infection because infected water then frequently enters the mouth, nose and eyes

During the Second World War the incidence of Weil's disease was altered in Amsterdam (Ruys, 1946) In the pre-war years (1935 to 1939 inclusive) only 13 per cent of 47 infections occurred in the fourth quarter of the year, but during the years of the war (1940 to 1945) 43 per cent of 83 cases occurred in the fourth quarter, owing to people falling into the canals during the 'black out' Also, in the hot summer of 1944 no case of Weil's disease was noticed in spite of much swimming, this was probably due to the high salinity of the water of the town and neighbourhood after the canals had been filled with brackish water Wolff and Ruys (1953) found that the number of cases notified in the Netherlands during 1934–39 inclusive was 531, compared with 276 during 1946–51 In Amsterdam, there was similarly a decrease from 185 to 111, this was made up by a fall to about two thirds the number of cases in the

second period in the group of water contact by swimming and fishing, and to about one half in occupational contacts and in water accidents The ratio of the numbers of infections to the numbers of accidents remained the same

Widespread infection of rats by *L icterohaemorrhagiae* was found as in other countries, and Klarenbeek (1938) found at Utrecht that 31 per cent of 182 dogs which had leptospirosis during 1933–37 were infected with *L icterohaemorrhagiae* A cat was suspected of being the carrier of infection to two bakers (van den Brekel, 1938) Klarenbeek and Winsser (1937) found that a pig was infected

In 1931 Klarenbeek and Schuffner (1933) isolated from a Dobberman pincher dog in Utrecht a new serotype, *L canicola* The first known human infections by this type were recognized in the Netherlands in 1933 (Dhont *et al*, 1934) and by 1948, 49 cases had been recorded (Roos *et al*, 1937, Schuffner, 1941, Minkenhof, 1948) Klarenbeek (1938) found that 69 per cent of dogs which had leptospiral infection were infected with *L canicola*, and Klarenbeek and Winsser (1938) made a full investigation of the disease in dogs The infection was diagnosed in a cat (van den Brekel, 1938)

Schüffner and Bohlander (1942 a & b) found *L grippotyphosa* as the cause of leptospirosis in two children who had been bitten by field mice (*Microtus arvalis*) They established that this animal is a carrier of *L grippotyphosa* and (1943) found 21 human cases in the Netherlands up to the end of 1942

Wolff *et al* (1949) reported the first known human infection by *L ballum* which occurred in a laboratory worker from a superficial scratch by an infected mouse

FRANCE

In 1883, Landouzy described an illness of two sewermen in Paris, which he called hepatic or bilious fever or hepatic typhoid fever, he ascribed the disease to an infectious agent caught in the sewers This was before Weil's publication in 1886

Infections of Allied troops by *L icterohaemorrhagiae* were detected in France and Flanders during the First World War (Stokes and Ryle, 1916 a & b, Dawson and Hume, 1916, Stokes *et al*, 1917, Costa and Troisier, 1916 c, Dawson *et*

al, 1917) Costa and Troisier (1918) emphasized the meningitic form of the disease During later investigations, Marie and Gabriel (1936) stressed that leptospiral meningitis is commoner in children than in adults and that epidemics with a seasonal incidence in the summer may be due to bathing In 1934 Erber reported that 286 infections of Weil's disease in all parts of France had been diagnosed at the Pasteur Institute, Paris, between 1923 and 1932

Infections by *L canicola* in France were first reported by Kolochine-Erber and Collombier (1945)

In 1940 von Hoesslin (1941) found infections of German soldiers in France by *L grippotyphosa*, and Buckland and Stuart (1945) detected two instances of infection by *L grippo typhosa* and one by *L sejroe* among British soldiers in Normandy Bastin *et al* (1953) found a boy who had acquired infection by *L grippotyphosa*, probably by bathing

Infection of pig keepers has been reported in several parts of France (Siguier and Poulet, 1947, Mattei *et al*, 1950, Boquien *et al*, 1950) In 10 cases the infecting agent was *L pomona* which has been found also, by serological test, to [illegible] 1950) In one [illegible])50) [illegible] *alis* in Strassburg, and Picard (1954) found infections by *L ictero haemorrhagiae* and *L ballum among rice workers in the* South of France

In French Indo-China, human infections by *L ictero-haemorrhagiae*, *L autumnalis*, *L kebler*, *L hebdomadis*, *L vauceli* *L canicola*, *L bataviae* and *L australis A* have been demonstrated (Ragiot and Delbove, 1934, Vaucel and Soulier, 1937, Kolochine-Erber *et al*, 1952, de Lajudie and Brygoo, *1953)*

Cases of Weil's disease were identified in French West Africa (Goez, 1933) and in French Morocco (Blanc and Delage, 1951)

BRITISH ISLES

During the First World War, British workers diagnosed Weil's disease by bacteriological and serological methods in British soldiers in Flanders (Stokes *et al*, 1917, Dawson *et*

al, 1917) The first case proved to arise in England was in a man infected by immersion in the Thames and was reported by Manson-Bahr *et al* (1922) In Scotland, the disease was found chiefly in coal miners in East Lothian (Gulland and Buchanan, 1924, Buchanan, 1927) Buchanan also demonstrated *L icterohaemorrhagiae* in rats in Scotland and in slime from a coal pit

Since 1934, evidence of the disease has been reviewed at various stages by Alston and Brown (1937), Broom and Alston (1948) and Broom (1951 a) During the recent years 1951–54 inclusive, the number of cases known in England and Wales was 98, 117, 118, and 125 respectively The death rate in this series was 15 per cent and was lower than in a previous series covering the years 1940–46 (Broom and Alston, 1948) in which it was 22 per cent

In Table X the occupational incidence is given of almost 1,000 cases reported in the British Isles from 1933–48 The proportion of coal miners and fish workers in the second series is less than in earlier surveys, because of improved working conditions, and greater in farm workers, probably partly by reason of better diagnosis The large 'miscellaneous' group mostly comprises occupations where contact with rat urine was likely In England and Wales, Broom (1951 a) found that in recent years 30 per cent of all infections have been in land workers

Coal mining formerly carried a much greater risk of Weil's disease in Great Britain than it does now In Table X, referring to infections during 1933 48, 14 2 per cent were coal miners, but in Broom's series 1947–50 the percentage was 4 Good accounts of the disease in coal miners have been given by Buchanan (1927) for the Lothians, Stuart (1939 b) and Sharp (1953) for the West of Scotland, Swan and McKeon (1935) for Northumberland and Sladden (1939) for South Wales Reasons for the recent reduction of infection include the decrease of the use of horses in the mines, improved water pumping and the closing of many 'drift' mines which enter the ground by horizontal or gently sloping roadways (Jenkins and Sharp, 1946)

Bathing and accidental immersion in canals, rivers and stagnant pools was the cause of infection in 19 per cent of the

1947–50 series in England and Wales In the hot summer of 1949 there were 33 cases, and in the cooler summers of 1948 and 1950 there were 15 and 16 Working in canals, gravel pits, watercress beds, ditches etc contributed 2 5 per cent to that series. Lyon (1956) detected 3 coal miners and 1 brickwork employee who were infected by bathing and not at their work places

In the fresh fish industry in Aberdeen, workers were found by Davidson and Smith (1936) to be frequently infected by means of rat urine which contaminated offal left in the fish cleaning sheds during the night (Fig 16) These investigators continued their reports for several years, and up to 1948 had diagnosed approximately 200 cases (Davidson and Smith, 1939, Smith, 1949)

Broom and Alston (1948) included 11 infections in men working at the fish docks of Hull and 2 at Grimsby Hampson (1946) found leptospiral antibodies in the serum of only 1 out of 116 fish cleaners at Grimsby, and he attributed this low incidence to the use of sea water in washing the fish and the tubs

Food handlers in shops, restaurants and hotels comprised 4 per cent in the 1947–50 series in England and Wales

The percentage of sewer workers among all infections discovered in England and Wales has fallen from 8 per cent in 1933–48 to 5 per cent in 1947–50 The number of cases fell sharply in 1940 and remained low during the war but has increased again since 1946 The most likely reason was the cessation during the war of extensive repairs of the sewers, which involve handling of broken infected brickwork Extensive destruction of rats in London sewers was begun in 1942, but has not been sufficient to prevent 5 to 10 cases a year among one to two thousand workers As would be expected infection has occurred more often early than late in a man's period of service in the sewers, in 19 instances the range of service was from 3 weeks to 20 years, but in 8 the period was less than one year and in only 2 more than ten years The disease has been studied in sewer workers in London (Fairley, 1934, Alston and Brown, 1935, Broom, 1951 a), Glasgow (Stuart, 1939 a), Aberdeen, Newcastle, Wolverhampton and Brixham

In Northern Ireland 24 instances of leptospiral infection were recorded in 1952 and 38 in 1953, these were almost all Weil's disease, a number of infections have been found in Eire (Broom, unpublished data)

Among animals, infection by *L icterohaemorrhagiae* has been found in Great Britain in rats (Coles, 1918, Stevenson, 1922, Broom and Gibson, 1953), dogs (Okell *et al*, 1925), a wild fox (Dunkin and Laidlaw, 1925) silver foxes (Smith, personal communication), pigs (Field and Sellers, 1951, Power, 1951, Nisbet, 1951), and rarely in cattle (Field, 1949, Field and Sellers, 1950)

The first human infection by *L canicola* known in England was reported by Baber and Stuart (1946) Broom (1951 a) recorded 70 cases in the period 1947–50 in England and Wales and 78 are recorded during the following four years 1951–54 Joe and Sangster (1951) recorded the first three cases known in Scotland, and Seiler *et al* (1956) and Lyon (1956) provided other examples A case has been detected in Northern Ireland (Kennedy, Crozier and Houston, 1953)

The infection of dogs is frequent as is shown by serological surveys Stuart (1946 a) in Scotland, Broom and MacIntyre (1948) in England and Broom (1951 b) in specimens from Eire have found evidence of past infection in 25 to 40 per cent of the canine population

Infection by leptospires other than *L icterohaemorrhagiae* or *L canicola* has not yet been contracted (apart from laboratory infection) in the British Isles Keal (1957) reported a case diagnosed serologically as being caused by *L grippotyphosa*, but the patient was an engineer who became ill a few days after reaching London by air from Malaya Broom (1951 a) tested sera from 642 patients suffering from aseptic meningitis for antibodies against *L bataviae*, *L grippotyphosa*, *L pomona* and *L sejroe* with entirely negative results, but found 17 sera with significant titres against *L icterohaemorrhagiae* and 8 against *L canicola*

AUSTRALASIA

In 1933 and 1934 outbreaks of an acute febrile illness occurred in the Ingham region of North Queensland, Australia Clinically

the affection resembled Weil's disease, and Cotter and Sawers (1934) isolated strains of leptospires from some of the patients, who were mainly employed in the sugarcane fields, and from rats caught in the locality By a serological study of these strains, and of others isolated during the ensuing period, Lumley (1937) found that two new serotypes, *L australis A* and *L australis B*, were involved In the same year Clayton *et al* (1937) reported the isolation of another new serotype which was named *L pomona* by Derrick (1942) and Johnson (1942) recorded a fifth, *L mitis* (=*hyos*)

TABLE XXXII

ANIMAL RESERVOIRS OF THE AUSTRALIAN LEPTOSPIROSES
(after Johnson 1950)

Clinical Type	*Serological Type*	*Animal Reservoirs*
Classic Weil s disease	*L icterohaemorrhagiae*	Imported rats (*R norvegicus R rattus*) Dogs
Canefields leptospiroses	*L australis A*	Native rat (*R conatus*)
	L australis B	Imported rat (*R rattus*) ? Bandicoot (*Isoodon* spp)
Mild leptospiroses	*L pomona* *L hyos* (*mitis*)	Cattle, calves Pigs Dogs

A full review of leptospirosis in Australia was written by Johnson (1950) He showed that 96 per cent of the disease has been found in Queensland, that 61 per cent of it was due to infection by *L australis A* and *L australis B* in the sugarcane fields of North Queensland, 33 per cent to infection by *L pomona* or *L hyos* in North and South Queensland and in northern New South Wales, and only a few per cent of the cases have been found in Victoria and Western Australia Infections due to *L icterohaemorrhagiae* were only 6 per cent of the whole Table XXXII gives the animal reservoirs of the serotypes, and Table XXXIII shows the occupations of the patients

Stevenson, Hayes, Ferris and Wellington (1953) gave sero-

TABLE XXXIII

OCCUPATIONS OF 187 PATIENTS WITH LEPTOSPIROSIS IN AUSTRALIA
(after Johnson, 1950)

Occupation	*Classic Weil's Disease*	*Canefields Types*		*Mild Types*		*Total*
	L. ictero-haemorrhagiae	L. australis B	L. australis A	L. pomona	L. hyos	
Dairy farmers and employees	0	0	0	47	5	52
Sugarcane farmers and cutters	3	38	6	0	2	49
Butchers	0	0	0	28	2	30
Miscellaneous outdoor workers (timber getters, farm labourers, fettlers)	6	9	3	6	1	25
Meatworks employees	0	0	0	10	3	13
Hotel, café, fish market employees	6	0	0	0	0	6
Sewer workers	6	0	0	0	0	6
Pig farmers and employees	0	0	0	6	0	6
Total	21	47	9	97	13	187

logical evidence of infection by *L pomona* or *L hyos* or both in 7 6 per cent of 422 abattoir workers in Melbourne, Victoria, and they found three cases of meningitis due to *L pomona* in Melbourne Derrick (1952) found *L canicola* in blood cultures of two patients in the same State

During recent investigations in the sugar growing areas further serotypes have been discovered In addition to *L medanensis*, this series comprises strains belonging to four apparently new serological types (Smith *et al*, 1954) Of these, the ' Robi

and the

type belo

(1956) showed that the ' Celledoni ' type is related to *L javanica* and *L poi*, and proposed for it the name *L celledoni* Two additional new serotypes, the 'Esposito' type and the 'Valbuzzi' type which are related to *L australis A* and *L grippotyphosa* respectively were later reported by Smith and Brown (1955)

North Queensland have

58 cases investigated from July to November 1954 In both papers *the relation of infection to heavy rainfall was emphasized* Experiments demonstrating that leptospires can survive in soil, *and later be washed out, were made by Smith and Self (1955)* Regarding the site of entry of the organisms Cotter (1936) stated that workers in the cane fields sustain numerous cuts and abrasions of the hands, wrists and forearms from the cane leaves and cut ends of the cane, and that soiling of these parts is unavoidable Rats urinate on the cane when feeding and also on the soil in the vicinity, and the most probable portal of entry of the organisms is through these skin abrasions Cane cutters frequently use poor footwear and some go barefoot, and abrasions of the feet and around the ankles may also permit entry of the leptospires

Measures which are used in the canefields for the prevention of infection are described in Chapter XIV

In Derrick's series, 120 patients were persons engaged in cane fields and 99 patients were not, the infecting serotype was accurately diagnosed in 208 cases, and eleven serotypes

were recognized in the numbers indicated—*L australis B* (58), *L australis A* (48) 'Kremastos type (22) Robinson' type (17) *L celledoni* (15) *L hyos* (15) *L canicola* (12) Szwajizak' type (=*L mini*) (9) *L medanensis* (4) *L pomona* (3) Doherty *et al* examined 52 rodents caught in or near cane fields Antibodies against *L australis* 4 were found in 11 out of 16 specimens of *R conatus* and 9 were urinary carriers Leptospires were not found in the kidneys of 32 specimens of *Isoodon obesulus* (bandicoots) but 10 had agglutinins against *L australis A* and 2 against serotypes of the Hebdomadis serogroup

L pomona infects pigs and also causes red water in cattle Sutherland *et al* (1949) were the first to isolate the serotype from the latter species It has also been isolated from a carrier dog (Mackerras 1954) *L hyos* is endemic among pigs and Johnson (1950) stated that there is serological evidence that it infects cattle and calves although it has not so far been isolated from these animals

In New Zealand Kirschner and Gray (1951) Kirschner *et al* (1952) and Faine and Kirschner (1953) recorded human infections by *L icterohaemorrhagiae* and *L pomona* and found the first named serotype in rats They obtained serological evidence of infection of calves and pigs by *L pomona* and a strain of this serotype was isolated from sheep by Hartley (1952) A human case of canicola fever was reported by West and Whitehead (1953)

Serological evidence of leptospiral infection of human beings in Papua and New Guinea has been found by Australian workers (Forbes and Wannan 1955)

DENMARK

Leptospirosis was first recognized in Denmark in 1934 when 14 human cases of infection by *L icterohaemorrhagiae* were found, and it was shown that 25 per cent of 93 rats were carriers of the organism (Zuelzer 1936 a & b) Later Ottosen (1941) cultured leptospires from 33 per cent of 685 rats An excellent, concise review of leptospirosis in 808 persons in Denmark was given by Borg Petersen (1949)

From 1934–48 infection by *L icterohaemorrhagiae* was found

in 254 persons, with a decreasing proportion of jaundiced and of fatal cases as the years went on There was a distinct, unexplained seasonal increase of the infection in August to December inclusive The patients were men in 86 per cent of cases

As well as rats, dogs were found infected—especially in rural districts

L canicola infected 95 of the 808 patients during 1934–48 and these infections were more frequent in the last three months of the year Men were infected in 57 per cent of cases, there were no fatalities and 13 per cent were jaundiced This serotype was cultivated from dogs (especially in towns) and some excreted it for several months

In 1937, Borg-Petersen and Christensen (1939) identified a new serotype, *L sejroe* Borg Petersen (1949) found that this organism is carried mainly by harvest mice (*Mus musculus* sp) There were 414 human infections with this serotype in 1934–48 including 198 during the year 1943 when there was a plague of mice Infection occurred in the fields and in houses in country districts, and 66 per cent of patients were males There were three fatal cases (0 75 per cent) and 13 per cent showed jaundice

Infection by *L grippotyphosa* was found in 15 rural male patients during 1940–48 without a fatality, the serotype was isolated from 22 per cent of 132 voles (*Microtus arvalis*)

L bataviae was diagnosed serologically in 16 male country dwellers without fatality during 1940–46, but the serotype was not isolated from man or animals

In 1942 Borg Petersen (1944 b) isolated from two wood mice (*Apodemus flavicollis*) a new serotype, *L saxkoebing* In 1943 he found two human infections by it in Denmark

L poi was diagnosed serologically in two cases in 1945 and 1946, and the serotype has been cultivated from man and from the wood mouse (*Apodemus sylvaticus*) and from a bank vole (*Clethrionomys glareolus*) (Fennestad, 1956)

In 1943, Borg Petersen (1944 c) isolated another new serotype *L ballum*, from a field mouse (*Mus musculus*) He found that its pathogenicity for young guineapigs was similar to that of *L icterohaemorrhagiae* (for 14 out of 16 animals died 7 to 12 days after inoculation) although jaundice was caused by the

new strain in only 7 out of 16 animals Human infection by this serotype has not yet been found in Denmark

Infection of pigs and cattle by *L pomona* was recorded by Borg-Petersen and Fennestad (1956 b) They discovered that these infections were present only in certain islands where there occurs a striped field mouse which is the chief carrier of *L pomona* in Denmark They found (1956 a, Fennestad, 1956) serological evidence of infection of cattle pigs and horses in Denmark generally, by several different serotypes, and conjectured to what extent abortion of cattle might be due to leptospirosis Fennestad gave a good summary of the serotypes found to infect man and animals up to that date

SWITZERLAND

Gsell (1936) reported the first recorded infections in Switzerland by *L icterohaemorrhagiae*—four proved cases and one suspected case

It is one of the distinctions of the Swiss workers—Gsell and his colleagues—that they have elucidated the leptospiral nature of swineherd's disease after it had been studied clinically and epidemiologically in Switzerland and Savoy since 1914 They have given very accurate accounts of the clinical features and epidemiological details of the disease in several papers and in Gsell's book 'Leptospirosen' (1952)

Gsell (1944) showed by serological and cultural methods that swineherd's disease is a leptospiral infection Examination of pigs in certain parts of Switzerland showed infection by *L pomona* to be common, and later it was found (Gsell and Wiesmann, 1948) that *L hyos* also infects them Swineherds are infected from pigs' urine by both of these serotypes, and the name swineherd's disease refers specially to disease caused by them Rarely, pigs and pig farmers in Switzerland are infected by other serotypes Epidemiological and clinical details of swineherd's disease are given on pages 145-8

Gsell (1946 b) showed that benign meningitis is caused in Switzerland by other serotypes than those of swineherd's disease, and divided this form of leptospiral infection into three epidemiological groups

1 Field workers	*mainly infected by L grippotyphosa.*
2 Swineherds	mainly infected by *L pomona*
3 *Canicola fever*	due to *L canicola*

Infections diagnosed 1943–46 in Switzerland were by the following serotypes

L pomona	76
L grippotyphosa	41
L sejroe	23
L australis A	11
L canicola	9
Undetermined	3
	163

In recording an attack of canicola fever in a veterinary surgeon, Gsell and Kanter (1945) *mentioned that, in the year* 1944–45, 114 cases of this infection were seen in dogs at a *veterinary clinic in Zurich, and that 59 died*

Heusser *et al* (1948) concluded from serological evidence that moonblindness or periodic ophthalmia, in horses, is leptospiral, and they incriminated *L grippotyphosa, L pomona, L australis, L sejroe* and *L icterohaemorrhagiae* in individual animals (p 251)

ITALY

During the War of 1914–18, a few cases of *Weil's disease* were proved among the soldiers on the Austro-Italian front (Moreschi and Carpi, 1916, Ascoli and Perrier, 1916, Sisto, 1917) It appeared that the leptospiral infections were only a small proportion of the cases of jaundice

Leptospiral infections have been found much more frequently in Northern Italy than in the rest of the country Austoni *(1953) recorded that during the previous 15 years* there had been diagnosed serologically at the Istituto Superiore di Sanità *di Roma 953 cases of infection by L icterohaemorrhagiae*, and 90 per cent of these were in Northern Italy (Venice, Lombardy, Emilia and Piedmont), 10 per cent in Central and only 3 cases in Southern Italy These 953 were 67 per cent of all lepto-

spiral infections diagnosed at the Institute in that period The Northern part of the country has the most rivers In August and September 1944, 17 British soldiers were infected by *L icterohaemorrhagiae* after bathing in the River Arno or its tributaries or in bomb craters, 5 died (Hutchison *et al*, 1946)

Infection by *L canicola* was diagnosed serologically in 54 persons by the Institute up to 1951 46 of these lived in Northern Italy (Austoni 1953) Babudieri and Castagnoli (1940) found this serotype in 3 per cent of 159 dogs in Rome

In 1938, Mino (1938 1939) and Babudieri (1938) separately published the discovery of leptospiral infection among ricefield workers in the valley of the Po in Piedmont and Lombardy in Northern Italy The two investigators believed that they had each found a new serotype, and they named them *L mitis* and *L oryzeti* respectively Gispen and Schuffner (1939 b) showed that these strains are *L bataviae* It has been reckoned that about one eighth of all leptospiral infections in Italy, and two thirds of those in Italian rice workers have been by *L bataviae* The most frequent carrier is *Micromys minutus soricinus* A detailed account of the disease in rice workers is given on p 163

Mino (1942 a) found serologically 3 instances of infection by *L grippotyphosa* among 255 leptospiral cases during 1937–41, and 8 more such infections were found serologically by Babudieri (Austoni, 1953) Mino also found evidence of the serotype in 1 *Arvicola* out of 21 tested it is voles of this sort that are the usual carriers of the serotype, but they are uncommon in Italy

A few human infections which were first thought to be by *L sejroe* (Mino 1941) are now believed to have been by *L saxkoebing* (Austoni, 1953)

Mino (1942 a) found a new serotype—*L poi*—in a rice worker, an animal carrier has not yet been found for it

Babudieri and Bianchi (1940) isolated strains from rice workers which were first named 'Mezzano strains' and later identified by Babudieri (1941) as *L pomona* Austoni (1953) stated that infections with this serotype have been found specially in the Po valley, in cheese factories, pig farms and in

some rice fields Infection by *L hyos* has been found serologically in about 20 persons in Northern Italy (Austoni, 1953) *L australis B*, also, has been found on rare occasions in rice workers (Austoni, 1953)

UNITED STATES OF AMERICA

Wolbach and Binger (1914) isolated from a fresh water pond in Massachusetts the first observed leptospire, which they named *Spirochaeta biflexa* Noguchi (1917, 1918 a) established the morphological similarity to one another of the *Spirochaeta icterohaemorrhagiae* of Inada, strains from British cases of Weil's disease in Flanders, strains from wild rats in New York City and Wolbach and Binger's *Spirochaeta biflexa*, and he suggested the name *Leptospira* for the genus

The first human infection known in the U S A was of a laboratory worker accidentally inoculated with *L icterohaemorrhagiae* in Albany, New York (Wadsworth, Langworthy, Stewart, Moore and Coleman, 1922) Molner *et al* (1948) collected records of 306 human cases of Weil's disease in the U S A up to 1945, these were found in many States of the Union but specially in cities such as New York (Tiffany and Martorana, 1942), San Francisco (Meyer, Stewart-Anderson and Eddie, 1939a) New Orleans (Bruno, Wilen and Snavely, 1943, Senekjie, 1944) and Detroit (Molner *et al*, 1948) Circumstances of infection were similar to those elsewhere and included the occupations of gardener, veterinarian, sewer workers (Meyer *et al*, 1939 a) and bathing (Havens, Bucher and Reimann, 1941) Ward and Turner (1942) found in Baltimore significant titres of agglutination of *L icterohaemorrhagiae* by the serum of 17 per cent of 75 poultry dressers, 6 per cent of 48 meat packers and none of 24 candy makers Stiles and Sawyer (1942) gave the significant occupation in 71 cases Among others, Beeson and Hankey (1952) recorded cases of meningitis due to *L icterohaemorrhagiae*, and Stiles Goldstein and McCann (1946) emphasized predominantly renal forms of the disease

Meyer *et al* (1938 a & b, 1939 a) revealed the first instances of human infection by *L canicola* known in the U S A, the patients were two veterinarians Rosenberg (1951) listed 11

American cases, diagnosed two more and gave a very good review of the subject Beeson and Hankey (1952) recorded 12 instances of the infection, and one or two cases each were reported by Rosenbaum (1946) Molner *et al* (1948) and Pearson and Hall (1952)

Meyer *et al* (1939 a) isolated 11 strains of *L canicola* from dogs in California, and Raven (1941) Tiffany and Martorana (1942) and Jones *et al* (1945) showed evidence of the infection of dogs in Eastern States

In many of these instances domestic or professional contact with dogs was found, but Cockburn *et al* (1954) traced infection of 24 people by use of a swimming pool (possibly contaminated by horses), and Williams, Murphy, McCroan, Starr and Ward (1956) believed that 26 persons were infected in a stream to which infected dogs cattle and swine had access

Morton (1942) first showed that the hamster (*Cricetus auratus*) is susceptible to infection by *L canicola* as well as by *L icterohaemorrhagiae*

Baker and Little (1948) isolated leptospires from dairy cattle in New Jersey, and these were identified as *L pomona* by Gochenour *et al* (1950) Later evidence of leptospiral infection of cattle, mainly by this serotype, was found in 19 States, Reinhard (1953 a) gave a full account of it and Morse (1955) estimated that it causes a loss of 100 million dollars a year Pigs in many States east of the Rocky Mountains have been found infected by Sippel and Atwood (1953) and very extensive infections of herds of pigs, with considerable damage to health was found in Illinois (Bryan, 1955 a) and elsewhere Roberts *et al* (1952) found this serotype in 2 out of 6 horses all of which had leptospirosis One of the two had periodic ophthalmia The relation of leptospiral infection, especially by *L pomona*, to periodic ophthalmia of horses has been investigated by Yager *et al* (1950) Woods and Davis (1950) and Bryans (1955) The presumptive evidence which they found for the relationship is given in detail in Chapter XVI

Beeson *et al* (1951) and Beeson and Hankey (1952) first found human infection by *L pomona* in the U S A in a patient with iridocyclitis, and in two other cases of meningitis Schaeffer (1951) *found that 50 young adults were infected with this* serotype after swimming in a stream about which cattle and

swine pastured Coffey *et al* (1951) found a swineherd infected, and Krouse and Sigel (1952) and Spink (1952) found an infection in a slaughterhouse worker and meatpacking worker respectively Larson, E (1953) recorded a case in a fisherman with a skin wound, who fell into a stream

Gochenour, Smadel *et al* (1952) showed serologically that Fort Bragg, or pretibial, fever is due to *L autumnalis*, 40 cases were believed to have occurred in the summers of each of the years 1942, 1943 and 1944

L ballum was found in 15 rural house mice and in an opossum in Virginia (Yager *et al*, 1953)

Infection by *L bataviae* was diagnosed serologically in a trapper (Gochenour *et al*, 1951) Spain and Howard (1952) diagnosed a human infection by *L grippotyphosa*

It is probable that *L sejroe* is responsible for some infections in cattle in four States (Yager, 1953)

Ovine leptospirosis, due to a serotype which they did not name, was described in 19 sheep by Beamer *et al* (1953)

It is seen that at present there is a very active phase of discovery in leptospiral diseases in the United States The veterinary work, including experimental and therapeutic study, is described in more detail in Chapters XVI—XVIII

SOUTH AMERICA

In Argentina the first two cases of Weil s disease confirmed by serological tests were reported by Miyara, Martinez Leanes and Elroy Funes (1935) Savino and Rennella (1944) demonstrated leptospires microscopically in the kidneys of *R norvegicus* and *R rattus* in Buenos Aires Strains isolated from rats, and later from human infections were at first considered by these workers to constitute a new serotype which they named *L bonariensis* Later however (1949 b) they showed it to be identical with *L icterohaemorrhagiae*

Savino and Rennella (1944) isolated two other serotypes from pigs and man, which they named *L suis* and *L hyos* respectively, and they also established the presence of infections with *L canicola* In further studies (1949 a) they showed that *L suis* is identical with *L pomona* Most of the human infections with *L pomona* were attributed to bathing in water

contaminated by pigs Babudieri (1951 a) and Savino and Rennella (1950/53) proved *L hyos* to be identical with *L mitis* Johnson (p 167) This serotype infects cattle and horses as well as pigs in Argentina

Anchezar Illa and Vivoli (1949) identified infection by *L icterohaemorrhagiae* in a veterinarian and a technician and found that nutrias were infected by this serotype

In Brazil *Piza and Gomes* (1930) identified a case of Weil s disease In 1950, Gomes Corrêa and Jordao reported the serological diagnosis of infection by *L icterohaemorrhagiae* in 44 out of 146 suspected cases, two strains of the serotype were isolated in culture Infection of dogs and rats by *L icterohaemorrhagiae* was shown by isolation of strains and by serological tests in Sao Paulo (Guida 1948 b 1949 a) A few human infections by *L canicola* have been found (Correa and Meira 1949 Gomes *et al*, 1950, Corrêa *et al*, 1954) and infection of dogs by the same serotype was also detected by Guida (1949 b)

In one instance a serological diagnosis of infection by *L australis B* in a ricefield worker was made by Correa *et al* (1954) Guida (1948 a) reported that he isolated leptospires from 3 out of 50 pigs the serotype was not identified but it was neither *L icterohaemorrhagiae* nor *L canicola*

ISRAEL

A considerable amount of infection by *L grippotyphosa* (p 151) has been found in Israel The first record extensive disease of cattle by *Bernkopf et al* (1947) a of human beings by the same writers and by others urine of cattle transmitted infection to human bein contact and more remotely by contamination of dr water (Jacusiel *et al* 1948) For a time strains isolated these infections were known as *L bovis* Olejnik Shneyerson (1950) reported that more than 1 000 growers were infected in a district where the rodent *guentheri* was the carrier of leptospires which they *L geffeni* but which were proved by van der H to be *L grippotyphosa*

Infection of five pigsty workers by *L pomona* was

Sandler (1949) van der Hoeden (1955 a & b) found infection of cattle, with some deaths, due to *L canicola* and caused by jackals which were proved to be carriers The same worker (1956) studied leptospirosis in pigs and its probable transfer from them to human beings

More detailed information about leptospiral disease in Israel is given in the sections of Chapter X and XI in which the serotypes mentioned above are detailed

APPENDIX

CULTURE MEDIA AND LABORATORY TECHNIQUES

CULTURE MEDIA

occasionally used, most workers find rabbit serum the most satisfactory The serum of certain individual rabbits may inhibit rather than support growth, so it is advisable to test the suitability of each rabbit's serum separately before a ' pool ' is formed Sometimes the inhibitory effect is associated with the presence in the serum of naturally occurring agglutinins, but we have also noted it in the absence of any demonstrable antibodies

Most media also contain peptone, and Witte brand is commonly recommended When this brand was not available in England we found that Fairchild, Difco proteose No 3 or Difco Neopeptone brands provided satisfactory substitutes Czekalowski, McLeod and Rodican (1954) tested a number of different brands and noted, on occasion, considerable variations in ability to support growth between different batches of the same brand It is advisable therefore to make preliminary tests on new batches of peptone before taking them into use

Growth is often improved by the addition of small amounts of haemoglobin To explain this effect Czekalowski *et al* (1954) postulated that leptospires, which as the same authors (1953) showed

The optimum temperature for growth of leptospires is 30° to 32°C, but cultures will grow satisfactorily at temperatures down to 25°C For isolating strains from suspected cases of leptospirosis the World Health Organisation Report (1956) recommended incubation at 37°C until leptospires are first seen in microscopic preparations from the culture, thereafter the cultures may be incubated at the lower temperature The reaction of the medium should be *p*H 7 2 to 7 6

When making subcultures it is advisable to inoculate the fresh medium with about one-tenth of its volume of old culture Strains vary greatly in the rapidity of their growth, and also in the density they finally reach As a rule a definite increase in numbers can be observed by the third to fifth day, and maximum growth is reached after five to nine days' incubation

A very large number of recipes for preparing media has been published and only a selection can be given We have limited our choice to those which we have found satisfactory, and to certain others which are either widely used or embody some unusual features

To obtain serum, blood is taken from the ear vein of suitable rabbits and allowed to clot The serum is pipetted off, inactivated by heating for half an hour at 56°C and passed through a Seitz filter After the serum has been removed, an equal volume of distilled water is added to the clot which is then allowed to stand for 15 minutes The haemoglobin solution thus gained is filtered and used as indicated in the recipes

VERVOORT'S MEDIUM

(Vervoort 1922, 1923 , Wolff, 1954)

This medium was f [illegible]
by several workers
serotypes of leptospi
preparation

1 Dissolve 1 g of Witte peptone in 1 litre of distilled water and [illegible]

2

3

4

5 phosphoric acid will give a better precipitate and a clearer solution after filtering

6 After cooling, filter the solution through paper and heat for one-half hour at 100°C

7 Distribute in small sterile bottles or tubes, 3 ml in each

8 Heat the containers for one half hour at 100°C

9 *Add 0 3 ml rabbit serum* with a trace of haemoglobin to each

10 Inactivate for one-half hour in a water bath at 56°C

11 Check sterility by placing the bottles or tubes overnight in the incubator at 37°C

KORTHOF'S MEDIUM
(Korthof, 1932)

This medium is basically similar to Vervoort's, and the recipe given here is the modification which we use

Peptone-salt solution

*Witte peptone	0 8 g
NaCl	1 4 g
$NaHCO_3$	0 02 g
KCl	0 04 g
$CaCl_2$ (hydrated)	0 04 g
KH_2PO_4	0 24 g
$Na_2HPO_4 2H_2O$	0 88 g
Double distilled water to	1,000 ml

Steam for 20 minutes, filter through double thickness Whatman No 1 paper, pour into 500 ml Erlenmeyer flasks Steam 30 minutes on 3 successive days, or autoclave at 10 lbs for 15 minutes The *p*H of this solution, which must be clear is approx 7 2

To 100 ml peptone salt-solution add 8 ml sterile inactivated rabbit serum and 0 8 ml sterile 'haemoglobin solution', mentioned above Distribute in hard glass test tubes in 10 ml amounts (aseptically) Incubate for 4 days at 30°C to ensure sterility

FLETCHER'S AGAR MEDIUM
(Fletcher, 1928)

Sterilize separately and then mix

Tap or distilled water	5 to 7 ml
Nutrient agar (2 5 per cent)	0 5 ml
Rabbit serum	1 0 ml

FLETCHER'S BROTH MEDIUM
(Brown, 1935)

Sterilize separately and then mix

Distilled water	3 ml
Nutrient broth	0 5 ml
Rabbit serum	0 25 ml

* Many other brands of peptone are equally suitable

Noguchi's Medium
(Noguchi, 1917)

Since this medium contains a small amount of agar, it does not evaporate so quickly in hot climates as more fluid media Sterilize separately and mix

Nutrient agar	*0 5 to 1 part*
Ringer s solution	3 parts
Rabbit serum	1 part
Citrated rabbit plasma	0 5 part

Tube in 3 to 5 ml amounts

[Noguchi recommends overlaying with liquid paraffin, but this is not necessary]

Stuart's Medium
(Stuart, 1946 b)

Prepare *M/10 stock solutions of the ingredients* (except glycerine) and mix together in the following proportions

Asparagine (dextro-rotatory)	2 ml
NH_4Cl	10 ml
$MgCl_2$	4 ml
NaCl	66 ml
Glycerin (A R)	1 ml
0 02% aqueous phenol red	*10 ml*
Distilled water	91 ml

[illegible]

hour at 60°C

Hindle's Medium
(Hindle, 1925)

Mix in a Petri dish a portion of human faeces the size of a pea with 20 ml of the water to be examined, and keep the mixture in the dark at 25° to 30°C Leptospires are generally found in 10 days and reach a maximum on about the 20th day

Saprophytic leptospires present in water will multiply in this medium, but the pathogenic serotypes will not do so

LABORATORY TECHNIQUES

Demonstration of Leptospires in Human Blood

Wolff (1954) stated that leptospires can be detected by direct dark-field examination of untreated human blood in only about 8 per cent of proved cases A considerable concentration of leptospires can often be obtained however by the method of differential centrifugation which was elaborated by Ruys (1933)

1 Prepare an anti-coagulant solution of 1 per cent sodium oxalate dissolved in phosphate buffer *p*H 8 1.
2 Add 4 5 ml of patient's blood (freshly drawn) to 0 5 ml of the buffered oxalate solution, and centrifuge for 15 minutes at 1,500 r p m
3 Place a drop of the clear supernatant fluid on a slide and examine by dark-field illumination
4 If no leptospires are seen, one or other of two alternative procedures may be carried out
   (*a*) Centrifuge the plasma for 20 minutes at 10,000 r.p m Carefully remove the supernatant fluid, and examine a drop of the sediment microscopically as above, or
   (*b*) If no high speed centrifuge is available, mix equal quantities of the plasma and phosphate buffer *p*H 5 3, centrifuge for 30 minutes at 3,000 r p m and examine the sediment

A word of warning is necessary to inexperienced workers regarding a possible source of diagnostic error when this technique is used When a drop of normal blood is examined by dark-field illumination one frequently sees thread-like filaments, probably derived from blood platelets or red blood corpuscles, which are about the same size as leptospires As they are constantly being bombarded by particles in Brownian movement, the filaments wave about in the field but they never show active motility No difficulty in differentiating these artifacts from true leptospires will be experienced by anyone who has compared the two side by side, but beginners are very liable to make a mistake (Kathe and Engelhardt, 1951)

The Isolation of Virulent Leptospires from Water

Bathing Method (Appelman, 1934, van Thiel, 1948 a)—The abdominal wall of a guineapig is shaved and scarified The animal is placed for an hour in the water, which is warmed to 30°C and which reaches the animal's flanks

x

CONTINUOUS *PERCUTANEOUS METHOD* (van Thiel 1948 a)—The [illegible] pig and is surrounded by a water jacket at 30 C A glass cy[illegible] open at both ends and with a *drainage tube at the lower end is fixed* to the abdominal wall and the water allowed to drip slowly through for 2 hours

CONTINUOUS SUBCUTANEOUS METHOD (van Thiel 1948 a)—The hair is clipped from the abdomen of a guineapig and the skin perforated on one side to admit a glass cannula A small opening is made on the opposite side and the skin freed from the abdominal wall by blunt dissection to form a subcutaneous tunnel between the two openings The animal is fixed to a board as in the percutaneous method and water from a funnel is allowed to flow slowly through the tunnel As Smith and Self (1955) pointed out the speed of flow can be conveniently controlled by means of a transfusion drip

AGGLUTINATION TESTS

PREPARATION OF FORMOLIZED SUSPENSION — Suspensions for agglutination tests are prepared from well grown cultures The density is judged by microscopical examination and it is not *customary to make an actual count of the number of organisms* present Sufficient neutral formalin* is added to give a final concentration of 0 2 per cent and the mixture left overnight The suspension is centrifuged at low speed to sediment any clumps which may be present and the supernatant fluid transferred to a fresh container

Some suspensions remain stable and sensitive for months but others develop a tendency to spontaneous agglutination after varying *periods of storage and must be discarded Positive and negative* control sera should be included in every series of tests

DILUTION SCHEME —A drop technique giving final dilutions of serum in a series 1/10 1/30 1/100 1/300 etc was developed by Schuffner and Mochtar (1927) and Schuffner and Bohlander (1939) and is illustrated in Fig 36 in which the circles represent depressions in a porcelain palette In the top left depression (A) place 2 drops of serum and 8 drops of diluent which may be distilled water normal saline peptone water *or culture medium Mix and transfer* 1 drop

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* *A stock solution of neutral formalin is prepared by the addition of excess* solid $MgCO_3$ Small quantities of the liquid are removed as required and filtered through paper before use

to D, 3 drops to B and 1 drop to C Add 9 drops of diluent to D, and repeat the procedure as indicated in the Fig, which also shows the number of drops of diluent and culture to be added to give the final dilutions The same pipette may be used throughout, provided it is washed with boiling water and cooled before each mixture in the series is made

Agglutinin-Absorption Test

In principle this test is the same as the agglutinin absorption test which has been used for many years in the identification and antigenic analysis of bacteria, and it was first applied to leptospiral investigations

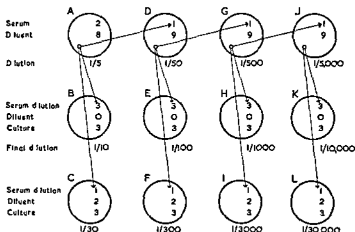


Fig 36

Scheme for preparing dilutions of serum and setting up agglutination or agglutination lysis tests in a palette

by Ruys and Schüffner (1934) The techniques described by different workers vary slightly in points of detail, but the method which we employ and which we have found satisfactory is as follows

A sufficient amount of neutralized formalin to give a final concentration of 0 2 per cent is added to 60 to 80 ml of a well grown culture in fluid medium The culture is distributed among four tubes and centrifuged for 30 minutes in an angle centrifuge The supernatant fluid is pipetted off, and a few drops are used to emulsify the precipitated leptospires in the 4 tubes The combined emulsions are made up to 19 drops and mixed with 1 drop of antiserum (or

patient's serum) diluted to a standard titre of 1/3,000 if the antibody content is above that level The mixture is incubated at 56°C for 1 hour and then left overnight at 5°C Next day the mixture, in a Dreyer's agglutination tube, is centrifuged for 20 minutes at 2,000 r p m, in a horizontal centrifuge, and the supernatant fluid is tested against formolized antigens for the presence of agglutinins As the serum has already been diluted 1/20, the first dilution in the test is 1/30 and is prepared by adding 4 drops of absorbed serum to 2 of suspension

If possible, absorption tests should not be made on sera with titres of 1/300 or less In a few fatal human cases however in which the question of industrial compensation was involved, we have obtained unequivocal results when the titres were as low as 1/100 In such cases 4 drops of serum were mixed with 16 of suspension, giving a final serum dilution of 1/5 instead of 1/20

Identification of Strains

The identification of newly isolated strains of leptospires is a lengthy procedure when circumstances make it necessary to compare their serological characters with a wide range of standard serotypes For example, Broom (unpublished) investigated more than 200 strains from Malaya, some isolated from human patients and others from domestic and wild animals When the work was begun, only *L icterohaemorrhagiae*, *L autumnalis* and *L pyrogenes* had been definitely identified in Malaya, although Fletcher (1928) also isolated other strains which he was unable to identify Broom tested each strain against antisera prepared against 32 serotypes, and evolved a technique with mixed antisera which considerably reduced the amount of work entailed

The method depends primarily on the observation that the presence of heterologous antiserum does not inhibit the agglutination of a strain by its homologous antiserum It is possible therefore to carry out a preliminary screening test with mixtures containing a number of antisera Experience showed that it was convenient to use mixtures of 5 or 6 antisera and to incorporate into different mixtures the antisera to serotypes which are related serologically By a careful selection of the serotypes represented in each mixture, one can often obtain a good indication of the group to which a new strain belongs from one test with a single dilution (1/300) of each mixture

The strain is next tested against a single dilution of each serum in the mixture or mixtures with which it has reacted The strain is then tested against a full range of dilutions of the antiserum or antisera

TABLE XXXIV

ANTISERUM MIXTURES FOR SCREENING LEPTOSPIRES

I	II	III	IV	V	VI
djasiman	andaman A	benjamin (2)	autumnalis (5)	australis B (4)	bangkinang (5)
hebdomadis (1)	australis A	hardjo (1)	canicola	ballum	celledoni
hyos	bataviae	mankarso (3)	javanica (4)	grippotyphosa	*Kremastos
icterohae- (2) morrhagiae	medanensis (1)	pyrogenes (4)	schüffneri (3)	poi (4)	sarmin
pomona	naam (2)	semarang	sejroe (1)	sentot	saxkoebing (1)
				wolffii (1)	mini (1)

The superscript numerals indicate serotypes belonging to the same serogroup
* Australian serotype not yet named

with which it reacted Finally the identification is confirmed in the normal manner by cross absorption tests

The six mixtures we use are listed above (Table XXXIV) The superscript numerals indicate serotypes belonging to the same serogroup

Mixed Antigenic Suspensions

When evidence of the presence of leptospirosis is being sought by means of a serological survey it may be necessary to cover a large number of serotypes in the tests The work can be reduced by using mixtures of antigenic suspensions Since only the homologous leptospires react with the antiserum, it is inadvisable to include more than three serotypes in one mixture, otherwise weakly positive reactions may be missed Formolized cultures must be used for this test, and the original suspensions should be of approximately equal density The mixtures should be centrifuged at low speed to sediment any small clumps which may be present.

Our own surveys have mainly been concerned with searches for possible carrier hosts, and the sera of such animals show relatively little reaction except with the homologous serotype In preparing the mixed antigens therefore antigenic relationships have been disregarded, and the serotypes are mixed, *in groups of three, in alphabetical sequence* The first mixture contains *L andaman A*, *L australis A* and *L autumnalis*, and so on

The preliminary screening test is made with a serum dilution of *1/100 because low dilutions of the sera of many apparently normal* animals agglutinate a number of leptospiral serotypes The same dilution is then tested against each of the separate components of any mixture with which it reacts Finally the titre of the serum is *determined against the homologous serotype*

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INDEX OF AUTHORS AND SOURCES

A

B

Y

Z

INDEX OF SUBJECTS

D

E

K

L

P

R

S

T

U

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